TECHNOLOGIES TO MAINTAIN BIOLOGICAL DIVERSITY

Volume II--Contract Papers

Part A: Plant Technologies

1. Status and Trends of Wild Plants
(Hugh Synge)

2. Status and Trends of Agricultural Crop Species
(Christine Prescott-Allen)

3. Diversity and Distribution of Wild Plants
(Marshall Crosby and Peter H. Raven)

4. New Technologies and the Enhancement of Plant Germ Plasm Diversity
(Tom J. Orton)

5. Plant Germplasm Storage Technologies
(Leigh Towill, Eric E. Ross, and Phillip C. Stanwood)

6. Technologies to Evaluate and Characterize Plant Germplasm
(Norm F. Weeden and David A. Young)

7. Assessment of Plant Quarantine Practices
(Robert P. Kahn)

8. Assessment of Market Institutional Factors Affecting the Plant Breeding System
(Frederick A. Bliss)

9. Historical Analysis of Genetic Resources Valuation
(Jean Pierre Berlan and Richard Lowentin)

10. Technologies to Maintain Microbial Diversity
(Jake Halliday and Dwight D. Baker)

December 1986

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Contract Paper No. 1

Status and Trends of Wild Plants

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This contract paper was prepared for the assessment of "Technologies to Maintain Biological Diversity." The views expressed are those of the author(s) and not necessarily those of OTA.

STATUS AND TRENDS OF WILD PLANT SPECIES

Paper Prepared for

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STATUS AND TRENDS OF WILD PLANT SPECIES

1. INTRODUCTION

Nearly half a century ago, Aldo Leopold told the story of the Silphium plant. He wrote of how every July he had watched a country graveyard in Wisconsin. In the graveyard was a small patch of prairie, a tiny remnant of the landscape when the graveyard was established in the 1840s.

> "Hitherto unreachable by scythe or mowers, this yard-square relic of original Wisconsin gives birth, each July, to a man-high stalk of compass plant or cutleaf Silphium, spangled with saucer-shaped yellow blooms resembling sunflowers. It is the sole remnant of this plant along the highway, and perhaps the role remnant in the western half of our county ...
>
> This year I found the Silphium in bloom on 24 July, a week later than usual; during the last six years the average date was 15 July. When I passed the graveyard again on 3 August, the fence had been removed by a road crew, and the Silphium cut. It is easy now to predict the future; for a few years my Silphium will try in vain to rise above the mowing machine, and then it will die".

This simple and clear example of how plants decline is typical of the inexorable, possibly accelerating, decline of plant diversity all over the earth except in those few diminishing areas of true wilderness. Some floras may be in greater danger than others, such as the floras of rain forests, islands, Mediterranean ecosystem and deserts, but there are few floras which are not in peril to some degree.

Yet it is difficult, in a sense impossible, to quantify this loss. Each case is an isolated event - the roadcrew mowing a country graveyard, the peasant farmer cutting a little more forest for his crops, the nomad cutting down one more tree to cook his evening meal. It means that the loss cannot be measured at one place and predicted at another; it is unlike, for example, changes in the atmospheric CO_2 content, which can be accurately quantified on the basis of relatively few measurements. All we know is that the loss is usually incremental, since in most areas the vegetation won't regenerate.

Plant conservationists face another major difficulty in trying to assess the scope of the problem: our knowledge of the c. 250,000 plant species of the world is very uneven. A few plants, notably the main economic crops, are known in great detail, whereas most tropical species are only known each from a handful of herbarium specimens, some collected many years ago. This enables the plants to be described, named and placed in the scheme of classification, but is insufficient to assess their survival prospects and conservation needs.

One reason for this imbalance is that about two thirds of the world's plants grow in the tropics - one third are in Latin America alone - but most of the botanists live and work in the temperate realm. As a result, temperate floras tend to be well known but low in plant diversity. In contrast tropical floras, especially in South America, still resemble a botanical frontier with thousands, maybe even tens of thousands, of species waiting to be discovered. Yet this is where the greatest losses of plant diversity are occuring. At the same time as scientists and decision-makers are coming to appreciate the significance of the biological diversity issue, the science of plant classification - taxonomy - is at a lower ebb than it has been since before Linnaeus, starved of funds, without proper training facilities and low in morale. The present condition of plant taxonomy is so critical to the conservation of plant diversity that it is the subject of a separate paper in this series by M.R. Crosby and P.H. Raven.

In essence, then, we cannot measure the decline in plant diversity directly. Rather we have to assess it indirectly, using such data as are available to extrapolate about trends and priorities for action. This paper outlines these methods and gives a very brief analysis of the results so far. From the conclusions drawn, recommendations are made for action.

2. PLANT EXTINCTIONS IN THE PAST

There has, it seems, been much less examination of extinction patterns in plants over geological time as compared to animals. Yet results of work by A.H. Knoll at Harvard University show some striking conclusions of great relevence to the conservation of biological diversity. The rest of this section is a very brief resumé of two of his recent papers - Knoll (1984) and Knoll (in press).

Mass extinctions have been clearly demonstrated to punctuate the evolutionary history of animals. There is, however, no evidence in the fossil record for similar global extinctions in plants. To explain this, Knoll argues that the situation is fundamentally different for plants. Plants may be more vulnerable than animals to extinction as a result of competition from newly appearing taxa and as a result of climatic change. But plants may be relatively resistant to extinction following catastrophic mass mortality or gross environmental damage. The reason is simple: most plants will regenerate from underground portions, such as stocks, bulbs, rhizomes, and many can survive adverse times in the form of seeds. Thus a short-term event, while decimating plant populations, may not lead to extinction. The reproductive profligacy of plants ensures their survival.

Knoll (1984) provides diagrams showing the number of vascular plant species, families and orders recorded per epoch in phanerozoic time (from quoted sources). At the species level, there is a steady increase, with only small dips in the Devonian and Triassic periods. It is important to note, however, that as the longevity of the plant species is generally shorter than the intervals of geological time, the graphs do not indicate general background extinctions, but presumably would show mass extinctions. For the dip in the Devonian, Knoll provides evidence for sequential replacement of one dominant group by another under the force of competition. He ascribes the dip in the Permian and Triassic Periods to high latitude warming and to increasing aridity in equatorial continental environments, combined with the rapid expansion of the conifers in the Permian. He suggests that global losses of plants at the Creataceous/Tertiary boundary were moderate and that the effect of the boundary events seem to have been minor, despite the strong evidence from geology and from the animal fossil record for the arrival of an asteroid at that time.

The logical conclusion of this fascinating work is that, unlike animals, competition and to some extent climatic change have been the main causes of plant extinctions until the era of man. There has been a steady accretion of plant diversity over geological time, with great bursts of evolution in the Carboniferous and Cretaceous Periods. Our generation is living through - and is causing - the first mass extinction event in the 4000 billion year evolution of the plant kingdom.

(I am indebted to Dr P.S. Ashton to drawing this work to my attention and to Dr Andrew Knoll for his kindness in sharing his research results, some still unpublished, with me.)

3. METHODS OF ASSESSING PRESENT DECLINE IN PLANT DIVERSITY

3.1 THREATENED SPECIES

Following the work of zoologists, much of the early work in plant conservation focussed on trying to identify threatened species, perhaps too much. At first sight the concept of a 'threatened species' looks simple, obvious even: it is a species that will become extinct rather soon if nothing is done. And, it is argued, the first priority is to prevent extinctions, to preserve the building blocks from which ecosystems and habitats can be reconstructed. In hindsight, botanical conservationists would have been more successful if they had been more orginal; only now are the key concepts and theories emerging.

How do we define a 'threatened species'? Many years ago, the Species Survival Commission of IUCN designed a set of criteria for defining the degree of threat to species; these have stood the test of time and have been used in virtually every country except the United States, where terminology and criteria have followed the non-compatible definitions of Endangered and Threatened in the Endangered Species Act of 1973. The IUCN categories, termed the Red Data Book categories, are defined in Annex 1; a booklet outlining their application with examples is available from IUCN.

Extinct and Endangered are obvious enough; in essence a Vulnerable species is one that is declining to the extent that it will become Endangered if nothing is done. The Rare species is one that is presently so rare that it could be eliminated easily, but is presently under no immediate threat and its population is more or less stable. The Indeterminate species is one which falls into one of these categories but where it is not certain which one. The slightly misleading use of English in these categories, regretted by some, has in my opinion been more than compensated by the strong impact of the words themselves.

In the discussions that follow the epithet "rare or threatened" is used for taxa falling into the categories Extinct, Endangered, Vulnerable, Rare, or Indeterminate. It is important to note that a species termed "rare or threatened" is not necessarily one on the brink of extinction, and that the statistics of Extinct and Endangered species, with the possible addition of Vulnerable species, may be more indicative of rapidly degrading floras than those for "rare and threatened" species.

Our experience has been that the categories must be subjective, as our information about threats is itself subjective. Quantification usually results in unfounded value judgements being made, e.g. that a pretty flower is worth 3 "threat points", closeness to a hydrodam 2. The results are rarely much use for assigning priorities for action: common sense provides a better guide.

How is a list of threatened plants prepared? In our view, there is only one way that works. First a desk researcher should make a list of all taxa occurring in the country other than those obviously common. The larger the flora, the more selective the search will have to be. For example, when preparing the European list in the 1970s, IUCN selected all single-country endemics, all border endemics, all species on national threatened plant lists available then, and all wetland plants as they were likely to be both widespread and threatened. Then the researcher must spend time with these botanists who know the flora in the field, sometimes with a life-time's experience, to ask simply which species on the list are under threat and rare within the areas they know. The information should be compiled and assembled under the guidance of a senior botanist experienced in the flora concerned; he or she will spot obvious errors.

3.1.1 PRESENT KNOWLEDGE ON THREATENED PLANT SPECIES*

There is now a very substantial amount of knowledge on threatened plants. It is mostly very recent: for example in 1970, only Belgium had produced a threatened plant list, only Ronald Melville was cataloguing threatened plants globally, and there was only a scattering of papers on plant conservation. In retrospect, it is clear that significance of the global decline in plant habitats had not been appreciated.

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* This section draws heavily on the introduction to a forthcoming book "Plants in Danger: What do we know?" (Davis _et al._, in press). The tables are taken from the book, which gives references to figures quoted.

Today, in contrast, almost all countries of the developed world have produced Red Data Books listing their threatened plants (see Table 1). For example, in Europe, all but five countries have produced Red Data Books or threatened plant lists, and those five, with the exception of Albania, are likely to produce something soon. In the United States, there is a mass of lists and reports covering both the nation and individual states: the situation is complex and rather untypical of other countries, but the profligacy of independent initiatives and activities gives perhaps a glimse of the situation in other countries in future; it is described in section 5, below:

TABLE 1

Selected countries or regions of the "North" with Red Data Books

Country/Region	Species	Rare & threatened Taxa	Extinct Taxa	Endangered Taxa
Australia	25,000	1716	117	215
Europe[1]	11,300	1,927	20	117
New Zealand	2,000	186	4	42
South Africa	23,000	2122	39	107
U.S.S.R.	21,100	653	c 20	c 160
U.S.A.[2]	20,000	2050	90	?

1. Excludes European U.S.S.R, Azores, Canary Islands and Madeira
2. Continental U.S.A.

Among the countries of the 'North', there are still some that have not yet produced such reports; the most prominent exceptions are shown in Table 2:

TABLE 2

Countries of the 'North' without national Red Data Books or threatened plant lists

Country	No. Species	Notes
Albania	3200	
Austria	3000	National list in prep.; many regional lists
Canada	3200	Several regional reports listing species likely to be rare, but without assessment of which are threatened.
Iceland	500	Unpublished list of 44 threatened taxa.
Israel	2300	
Italy	5000	Last national list in 1959, examples only.
Japan	4000	Very poor information at present.
Portugal	2500	National list in prep.; various unpublished material.
Saudi Arabia	3500	

In North America, 10-11% of the taxa have been listed. The figure is rather higher in Europe, probably because of the combination of extensive threats to vegetation in the northern, industrialised countries, and the high degree of plant endemism in the southern Mediterranean countries. Coverage of the U.S.S.R. is patchy, despite a plethora of literature, and the emphasis is on threatened economic, specifically horticultural, plants.

For the Southern Hemisphere, there is a list for South Africa, although heavily weighted to the Cape. A good list is available for Australia, now in its third version, though it is known to be incomplete for the fast disappearing Queensland rain forests and for the extraordinarily diverse flora of Western Australia; botanists estimate that as many as 7000 plant species await discovery in Australia, mainly in the western region. In temperate Latin America, there is a list for Chile, but not yet for Argentina.

Of all countries, the problem of threatened plants has perhaps been best documented in New Zealand, due to the efforts of Dr David Given. First to appear, in 1976, was a register - or list - of 314 taxa under consideration for threatened status (Given 1976a). Then, in 1976-1978, a set of loose-leaf sheets was issued; each sheet covered an individual species, with emphasis on the exact localities for each species and populations in each locality. This was not a public document, but designed to provide the practising conservationist with the information needed on the most critical threatened plants. This was followed by a popular, illustrated book on conservation of the New Zealand flora (Given 1981a), an official Red Data Book, covering plants and animals (Williams and Given, 1981) and a paper describing the whole documentation process (Given, 1981b).

Within these regions, the highest percentages of rare and threatened species are from those areas with a mediterranean climate - the Mediterranean basin countries themselves, Western Australia, the Cape of South Africa and California. Raven (1976) estimates that these regions contain at least 25,000 plant species; a high percentage of them, maybe as many as half, are narrow endemics, and it is these plants, mostly in the Rare or Endangered categories, that dominate the threatened plant lists for U.S.A., Europe, Australia and South Africa

In addition, threatened plant lists and Plant Red Data books have been prepared for many islands. For example, the Canary Islands are well covered by the list for Spain (Barreno et al., 1984), a Red Data Book for Mauritius, sponsored by IUCN/WWF, is in preparation and several lists have been prepared for the species-rich islands of Hawaii. Emphasis, however, has been more on listing the endemics and assigning threatened categories rather than preparing Red Data Books as such. Nevertheless they show convincingly the very high degree of species endangerment on islands, especially tropical islands.

The most important islands for plant conservation are those with large endemic floras. Those with over 1000 endemics are listed in Table 3. Four of them - Madagascar, Hispaniola, Cuba and New Caledonia - are very ancient land masses, unlike most oceanic islands which are of more recent geological origin. These islands contain remarkable floras that are very distinct, often isolated biologically and relicts of floras no longer seen today. This is demonstrated by the high rate of endemism among genera and even families. In all of them the vegetation is acutely threatened, but only for Cuba and Hawaii are there assessments of which species are at risk. More detailed survey and assessment of the conservation status of these floras is an urgent world priority.

TABLE 3

Oceanic islands with over 1,000 endemic plant species

Country	Size of flora	Endemics
Madagascar	10,000-12,000 spp.	Around 80%
Cuba	6,000-7,000 spp.	3,000-4,000
Hispaniola	5,000 spp.	1,800
New Caledonia	3,250 spp.	2,474
Hawaii	2,600 taxa	c. 90%

Hispaniola comprises the nations of Haiti and Dominican Republic.
Excludes Australia, New Zealand, Borneo, New Guinea.

There are, of course, many other islands with rich floras, although none except the five listed above have over 1000 endemics. Here, paradoxically, data are usually more complete. Some examples are given in Table 4, using the IUCN Red Data Book Categories to define the degree of threat. The following table (5) lists those islands with between 50 and 1000 endemics for which no assessement of the degree of endangerment of the flora has been made. It is reasonably complete.

TABLE 4

Endemic vascular plant taxa from selected oceanic islands

50-1000 endemics, over 75% of the flora assigned to categories

	Ex	E	V	R	I	K	nt	Total	Rare or threatened
Azores	1	-	5	18	6	11	14	55	30 (55%)
Canary Islands	1	126	119	132	5	26	160	569	383 (67%)
Galapagos		9	15	111	15	2	77	229	150 (66%)
Juan Fernandez	1	52	32	9	1	17	6	118	95 (81%)
Lord Howe Island	-	2	10	58	3	-	2	75	73 (97%)
Madeira	-	17	30	39	-	22	23	131	86 (66%)
Mauritius	19	65	35	39	14	69	39	280	172 (61%)
Seychelles	-	21	35	15	2	17	-	90	73 (81%)
Socotra	1	84	17	29	1	2	81	215	132 (61%)

Similar data exist for Puerto Rico (234 endemics) but are not yet converted to IUCN criteria. Figures for Seychelles are for the Granitic Islands only, and so exclude Aldabra.

TABLE 5

Islands with significant endemic floras (50-1000 endemics) not yet assessed for species endangerment

Island	No. endemic taxa
Jamaica	c 910
Taiwan	c 900 [1]
Sri Lanka	c 900 [1]
Fiji	c 700
Caroline Islands	293 [2]
Trinidad and Tobago	215 [1]
Ogasawara-Gunto	151 [1, 2]
Réunion	c 150 [1]
Vanuatu	c 150
Tubuai	140 or less
Comoro Islands	136 [4]
Bahamas	121 [1]
Sao Tomé	108
Marquesas Islands	103 [1]
Samoa	? 100 or more[3]
Cape Verde	92
Mariana Islands	81 [2]

Notes: 1. Some partial assessments of threatened species
2. Omits monocoyledons
3. Covered as American Samoa and Western Samoa
4. From a checklist published in 1917

A number of tropical countries have prepared or are preparing threatened plant lists, despite the difficulties (see 3.1.2, below). Among the most prominent are:

Brazil	Listing project sponsored by IUCN/WWF.
India	Several lists prepared, covering in total c. 900 threatened species.
Nepal	National Plant Red Data Book, funded by WWF-US.
Pakistan	Identification of threatened plants is part of the WWF-Pakistan Plants Programme.
Peninsular Malaysia	Database on threatened plants being created by the Malayan Nature Society.

In middle America (Mexico to Panama), an IUCN project has prepared a threatened plant list for the region, using where possible the floristic accounts prepared for the forthcoming Flora Mesoamericana. 7622 single country endemics were identified. Of these only 2467 are sufficiently well known for Red Data categories to be assigned. Overall 1620 species were identified as rare or threatened but this is undoubtedly a major underestimate.

As outlined in Section 3.1.2, it is rarely possible to identify threatened species, especially in tropical forests, until it is too late. All the evidence, however, is that plant extinctions are highest in precisely these areas. Latest FAO figures show that tropical closed forests amount to 56% of their potential extent and are being clear-cut at 1.1% each year. The reality is worse than these figures imply because the FAO figures only cover outright destruction. Large additional areas are being degraded by extraction of timber and other products. Tragically, any destruction of primary lowland forests, at least in Asia, results in a loss of plant species diversity. (This is not always the case for fauna, especially mammals.)

Some examples, too, show the extent of the loss. The natural vegetation of Madagascar, lowland western Ecuador and the Atlantic forests of Brazil have been reduced to less than 10% of their extent 50 years ago. Each may have had of the order of 5000 species, many of which will now be extinct (Raven, in prep.). Comparing regional data on floristic richness and information on forest loss, leading botanist Peter Raven estimates that 60,000 plant species - a quarter of the world's total diversity - are at risk of extinction in the next 30-40 years.* This estimate, although crude, was agreed as the best to follow by the IUCN/WWF Plant Advisory Group (November 1985) and is lkely to be accepted by IUCN. This author believes it to be substancially correct.

3.1.2 LIMITATIONS OF DATA ON THREATENED PLANT SPECIES

The concept of a species threatened with extinction is a simple one, but it is proving very difficult to identify threatened species in many parts of the world. As outlined above, most threatened species recorded so far grow in the 'North', whereas most of the world's plants are in the 'South', where the threats are also most acute too. Raven (1976) said, "Realistically we have no opportunity to determine with any degree of precision which plants are rare or endangered in most of the tropics before the whole ecosystems in which they exist become depleted or even cease to exist". Why is this so? Because of a combination of the speed at which tropical habitats are being destroyed and the lack of knowledge about their content. Data on the loss of habitats is well documented elsewhere; below I outline some of the botanical factors involved.

1. Many plants have not yet been discovered and described by scientists. This certainly amounts to thousands, possibly tens of thousands, of species, the greatest proportion from Latin America. Every year thousands of new plant species are described.

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* This is only approximately comparable with the number of species that would fall into the IUCN Red Data Book categories if all the data were available. The Red Data Categories, designed for a species by species approach, relate to threats today, whereas the Raven estimate relates to threats that seem likely on present trends over the next 30-40 years. On the other hand, it is likely that many, perhaps most, of the predominantly mediterranean-climate species presently categorised as Rare will survive the next 30-40 years. These two factors probably cancel each other out. If finance could be found, IUCN is keen to do a major project to estimate more precise extinction rates, country by country, and from this to prepare an analysis of the plants at risk in vulnerable but as yet unquantifiable floras. (Estimated cost US$ 100,000 - 200,000.)

2. Even if discovered, the information on each species is so small that it is not possible to assess chances of survival. Numerous tropical plants are only known from a handful of herbarium specimens, often collected many years ago and frequently poorly documented. Whether the plant is common, even dominant over a large range, or extremely rare, is not known.

3. There is great difficulty of identifying tropical plants, especially in rain forests. To the amateur, the plants all look so alike, yet 10-ha of Malaysian jungle may contain over 700 different trees (P.S. Ashton, pers. comm.). The sheer difficulty of tropical botany is often forgotten; whereas in New England, a good amateur can easily identify virtually all he or she may see, few expert botanists can identify more than a small fraction of a tropical forest flora, at least without reference to an herbarium.

4. The distribution patterns of many tropical plants. The rare species in regions of Mediterranean climate and on islands tend to be point endemics, that is plants only known from one small place, be it a cliff or scree or mountain top. Here the threat to a site can be equated to the threat to a species. Build a ski resort on the mountain top or remove the cliff and the plant will disappear. But in the tropics, especially tropical rain forests, many plant species tend to have a very scattered distribution. The natural corollary of so many tree species in one 10-ha plot is that there can only be one or a few individuals of each species within that area. So the plants tend to be thinly scattered over a very extensive range. (Point endemism occurs too, especially in the most species-rich areas, e.g. coastal NW Borneo, SW Sri Lanka, Guyana Highlands, Colombian Andes.) If part of the forest is to be cut down, it is usually not possible to say which of the numerous widespread and scattered species would become extinct and which would not. Furthermore, Raven (1976), citing Elton (1975), points out that tropical plants appear to be overwhelmingly outbreeders, so that their reduction to small populations may cause their demise.

This is not always true. One exception is the Sinnaraja Forest in Sri Lanka. It is the only extensive remnant of lowland rain forest on the island, but has been reduced from 24,000 acres to under

14,000 acres in recent times. Yet it is the only locality for about half of the 800 or so plant species endemic, i.e. confined, to Sri Lanka. So very clearly, if the remaining forest is felled, about 400 plant species will become extinct.

Even if threatened species can be identified, what can governments in tropical under-developed countries do with the results? A set of reserves to cover the hundreds, in most cases thousands, of species involved is barely practical. Protecting each one by law is usually equally futile. One answer is to concentrate on sites where diversity and/or endemism is greatest, and this is the subject of section 3.3.

3.2 STUDIES ON THE DECLINE OF ECONOMIC SPECIES

Often the most detailed information on the decline of diversity in plants comes from data on economic plants. This is often virtually the only source of data on the decline of variation within a species. Economic plants, too, tend to be over-harvested, which, combined with loss of habitat, leads to genetic erosion.

Of course some of the c. 15,000 species identified as threatened worldwide are themselves species of economic value or of potential use of man. Examples include the Yeheb (Cordeauxia edulis), a critically endangered bush from the drier parts of Somalia and Ethiopia, whose nuts used to be a major source of food in the past. Devastation of the habitat has removed this life-saving plant. Other examples among the crop relatives are the olive relative Olea laperrinei and the pomegranate relative Punica protopunica.

Far the best documented are of course the status and trends of agricultural crops and their relatives, but even here the data are mainly circumstancial and anecdotal, according to the separate paper by C. Prescott-Allen in this series. A main conclusion of that paper is that there are no organized data sets sufficient to plan in situ conservation programmes. This is equally valid for the other, much more numerous economic species, to which it may be added that at first sight data appear insufficient for planning ex situ conservation programmes too.

A major problem is the lack of an accepted international list of economic plants as the basis for selective action. Although there are plenty of data on what individual plants can be used for - and most plants are good for something - it is more difficult to know which are the ones that really matter both for local economies and for world trade. Only the users of these plants can answer this question. Some components of the list already exist, e.g. ethnobotanical plants from South America (see below) and a list of priorities for forestry species compiled by FAO. To fill this gap, IUCN plan to prepare such a list, using consultants from each of the major sectors (e.g. forestry, medicinal plants, forages, vegetables, pulses, fibre crops and so on.) The IUCN/WWF Plant Advisory Group, a panel of leading botanists, will play a major role in steering the project and defining the criteria to be adopted for inclusion in the list. It is anticipated that the resultant list will cover about 5000 - 10,000 species. Funding of around US$ 100,000 - 200,000 is being sought.

The list can then be used for selection of priorities, for assessment of genetic erosion and for planning an effective programme of in situ and ex situ conservation, following the more detailed work done on the 100 or so major crops by FAO and IBPGR.

It is clear in hindsight that botanical conservationists have been far too slow to give adequate weight to economic plants. All too often, economic or useful plants have not been singled out for special attention. In this, plant conservationists have missed perhaps the most vital and easily justifiable part of their mission. Although efforts are now being made to correct this imbalance, far more needs to be done.

However, it is not the subject of this paper to outline the various types of other economic plants nor is their space to detail the data available on the uses of plants. Below we briefly comment on the status of two sets of useful plants - forest trees and medicinal plants.

3.2.1 FORESTRY TREES

Despite the great importance of forestry worldwide, there is no systematic analysis of loss of diversity in forest provenances, although there is an extensive literature of studies of individual trees. The list of the 59 woody species in The IUCN Plant Red Data Book has been widely used, but the species were only a selection from available data to illustrate the threats involved, and were not the most important ones. The FAO Forest Resources Division is preparing a Databook on Endangered forest Tree Species and Provenances, which it intends to publish in late 1985; the book contains sheets on 81 taxa, some of which, like Diospyros hemiteles, are Endangered as species, this one reduced to a single tree, and others, like Juniperus procera, certainly not threatened as species, but subject to genetic erosion in part of their ranges. The selection still shows signs of confusion between botanical lists of threatened species, and lists of important timber trees. For its part, IUCN has offered to prepare a list of all known threatened trees of the world for FAO, but this offer has not been accepted.

Examples of commercial extinction in trees are hard to find, though the demise of true mahogany has been well documented (Knees and Gardner, 1983). As Roche and Hamann (in prep.) point out, programmes for selection and breeding of tropical trees are virtually non-existent, except in a few cases, yet it is certain that genetic resources of useful timber trees in the wild are being continually eroded. Though little is known of the genetic resources of trees in the scientific world, local people are often extremely knowledgeable on the value and uses of the trees around them. This knowledge is for the most part untapped by conventional forest research, and is rapidly disappearing.

3.2.2 MEDICINAL PLANTS

A most acute failing is the lack of data on the decline of medicinal plants. We are told that 70-90% of rural populations in developing countries depend on traditional health remedies and that most of these involve medicinal plants, many collected from wild populations. For example, of the c. 2000 drugs used in the system of Ayurvedic medicine in India, some c. 1500 come from plants. Furthermore many prescriptions in developed countries were initially developed from plant materials, now cultivated rather than collected from the wild, and other medicines, although synthesised, are based upon chemical information discovered in plants. For example, vinblastin from

Catharanthus roseus gives leukaemia sufferers a good chance of remission for the first time; gossypol from cotton, which is used to treat bronchitis in Chinese folk medicine, may be a male contraceptive. (This paragraph mainly from Prescott-Allen, 1982; Myers, 1983).

New discoveries of medicinal plants tend to come from plants already used by tribal people. Therefore more emphasis need to be given to cataloguing the use of plants by tribal people before it is too late; here is a case where the knowledge is disappearing even faster than the plants. At present, M. Plotkin, under the aegis of IUCN and with Professor R.E. Schultes at Harvard, is preparing a catalogue of over 1000 plants used by Indians in Latin America; similar efforts are needed to apply his methodology in other parts of the world.

It is also vital to undertake more systematic surveys on the decline of medicinal plants, so they can be propagated and bulked up in medicinal plant gardens, and the genetic diversity of the wild populations conserved. A truly horrendous example is given by Gupta and Sethi, 1983: Dioscorea deltoidea is a very important plant in the Himalayas for its tubers contain a high concentration of diosgenin, a cortico-steroid used in the production of steroid drugs, such as anti-inflammatory agents, anti-cancer agents, oral contraceptives and anabolic steroids. Whereas early collections found tubers with 6% or more diosgenin, recent searches found no tubers with diosgenin content higher than 1%; the high-yielding genotypes had been exterminated by commercial herb collectors. Gupta and Sethi also list a number of medicinal plants from the Himalayas that are endangered as species - a good example is Coptis teeta, reduced to a handful of plundered plants.

3.3 STUDIES OF PLANT SITES, THEIR ENDEMISM AND DIVERSITY

Most botanists today feel the best way to save most tropical plants will be to preserve relatively few large areas of intact vegetation. Experience has shown that large areas are easier to manage in the tropics than small areas, which rapidly get degraded without constant supervision. It is also clear that small isolated sites of tropical forest will not persist and regenerate in the way that similar paches of temperate forest do.

It is certainly easier to assess which these sites should be than to identify threatened species. In Ivory Coast, for example, the only large extant piece of native forest remaining is the Taï National Park, nominally covering 330,000 ha; it has been steadily reduced by illegal and rampant logging, organised poaching and panning for gold. With over 150 plants endemic to the park, it is an obvious choice for conservation action.

In other cases the destruction has gone so far that the selection is simply of what forest remains. Perhaps the best example here is the Brazilian Atlantic forests, believed by many to be the most endangered rain forests in the world. The best estimate is that only 1-2% of the Atlantic forest is in a condition worth saving. Only tiny fragments now remain and opportunities for conservation are rapidly being closed. These forests, which once stretched in a narrow strip from the state of Rio Grande del Norte to Rio Grande do Sul, are far apart from the Amazon forest block and have a different set of species, with a high percentage of endemics, both plant and animal. Today the Brazilian conservation authorities, with substancial support from IUCN/WWF, are struggling to protect those areas still intact as reserves.

An advantage of the sites approach is that large protected areas offer many other benefits besides conserving valuable plant species. Enlightened park chiefs are changing their tactics, understanding that in developing countries meeting human needs has to be the primary goal. Rather than "set land aside", they want to protect it so that the benefits continue to radiate out into the surrounding countryside - migrating meat for the pot, fresh water in the streams, markets for local handicrafts. A key concept is that of the buffer zone, a broad and fuzzy edge between the park and the neighbourhood; here people could get firewood and wild fruits, graze limited numbers of cattle, and perhaps gather medicinal herbs - many Africans feel that sustaining supplies of wild medicinal plants is the best justification of all for protected areas.

In many cases, the protected areas are critical watersheds, where clearing of vegetation can do great harm to the supply of fresh water to neighbouring cities. The vegetation on the Ulugurus, for example, is very important for the water supply to Dar es Salaam, the largest city in

Tanzania and one of the fastest growing cities in Africa. The vegetation is extremely rich with several endemic genera and around 100 endemic species (Polhill, 1968), yet is declining fast (J. Lovett, pers. comm.) In recognition of the watershed argument, a project sponsored by the World Bank to develop rice production in Sulawesi contributed substantial sums to the development of the large Dumoga-Bone National Park, a large piece of plant-rich forest along the ridges above the rice fields; in this way the long-term future of rice production is assured from erosion.

Many of the sites where most plants can be saved are already National Parks but only in name. Areas of hundred of thousands of hectares may have a park staff of a handful of people, mostly untrained, and no equipment. Here the great need is for consolidation: technical aid to help develop management plans, training for staff, and equipment. In many cases, institution-building is needed too as how can you manage a big park without a functioning parks department? (The issues described in this section so far are the subject of a paper in this series by J. Thorsell.)

A first selection of key plant sites worldwide was made by the author when, in 1982, he was invited by the World Wildlife Fund to develop plans for how they could achieve the most plant conservation with the c. US$3 m they hoped to raise in their Plants Campaign during 1984-85. The programme devised had two themes: a set of strategic projects to make plant conservation a more accepted part of conservation as a whole, and more traditional field conservation projects, usually to establish or consolidate protected areas, in as many plant-rich sites as possible.

The initial analysis, widely circulated in late 1982, contained c. 50 sites; this was since reduced to about 20, because it was felt IUCN and WWF had neither the finance nor the administrative capacity to cover more at that time. Sites within the territories of WWF National Organisations (NOs) were omitted too, because each WWF NO develops and manages projects in its own country independently rather than through the IUCN Programme. The sites are listed in Table 6. Just over a year into the campaign, over US$ 1.6 m had been committed, mostly to site conservation. It is likely that the full programme will be carried out more or less on schedule.

Encouraged by this, IUCN is preparing a Plant Sites Red Data Book; this will contain accounts of c. 150 botanical sites indicative of those in greatest need of protection, and where plant species diversity and/or endemism is particularly high. It is not intended to be a comprehensive account of all sites in danger, but rather an indication of those where most plants could be saved. The intention is to present it to conservation organisations and aid agencies, to guide the aid they offer. The book will provide perhaps the most cost-effective and practical way to conserve plant diversity. (Whereas the selection of the sites and the production of the book is funded, the next step of a feasibility and costing study on its implementation is not.)

TABLE 6

Plant Sites selected for technical aid under the international component of the IUCN/WWF Plants Programme

Africa	
Cameroun	Mt Cameroun, lowland forest sites - Dja (540,000 ha), Korup (90,000 ha) and Pangar-Djerem (480,000 ha)
Ivory Coast	Taï Forest
Liberia	Sapo National Park (130,700 ha of Guinean forest)
Madagascar	Various sites in the forest belts with emphasis on Ankarafantsika and Ambonitantely
Mauritius	Endemic flora rescue programme; establishment of small reserves
Niger	Aïr and Ténéré National Nature Reserve (6.4% of Niger)
Tanzania	Montane Forests (Usambaras, Ulugurus, Uzungwas)
Asia	
India	Support to plant conservation, especially proposed Biosphere Reserves in Assam and for Nilgiris
Indonesia	Selected reserve sites, predominantly lowland forests in Kalimantan and Irian Jaya
Malaysia	Selected forest sites in Sarawak
New Caledonia	Sites to be selected
Sri Lanka	Sinnaraja Forest (see text)
Central and South America	
Brazil	Selected sites in the Atlantic Forest (see text)
Peru	Manu National Park (1.5 m ha)
Costa Rica	La Amistad National Park (192,000 ha), Tortuguero NP
Honduras	Rio Platano Biosphere Reserve (350,000 ha)
Chile	Juan Fernandez Islands
Ecuador	Galapagos Islands
Atlantic Islands	
Azores, Canary Islands and Madeira	

Note: This omits sites in WWF NO countries; these include Australia, Canada, Japan, New Zealand, Pakistan, South Africa, United States and most western European nations.

3.4 STUDIES ON THE DECLINE OF HABITATS AND BIOGEOGRAPHICAL STUDIES

In theory, data on loss of habitats could be the best clue to loss of plant diversity. If we know which habitats are richest in plants and where the vegetation is disappearing most rapidly, we can assess which areas should be priorities for action. In a sense this is implicit anyway in the previous approach, on selecting key plant sites for conservation.

Information on the decline of habitats worldwide is not outlined here as it is the subject of another paper in this series. Nor is the author presently qualified to describe and comment on the various techniques involved, especially satellite imagery. Suffice it to say that at international level, this will be the main focus of the GRID database (an offshoot of UNEP, to be based in Geneva) and that presently, under UNEP aegis and co-ordination, IUCN holds data on the decline of wetlands (with IWRB), on coral reefs and on island habitats (with ICBP); FAO holds data on tropical forests and ECE on temperate forests.

Two international projects, however, should be mentioned:

1. A Strategy on Botanical Inventory in Tropical Forests, being prepared by David Campbell, funded by WWF-U.S. and co-sponsored by the Arnold Arboretum, the Missouri Botanical Garden and the New York Botanical Garden. Individual experts have been asked to contribute chapters for each country with tropical forests, outlining the extent of botanical exploration and recommending priorities for action. Perusal of an early draft has shown a massive account of the vegetation of those countries and a lot of data on botanical knowledge, but a true strategy is still a long way off.

2. IUCN's Conservation Monitoring Centre is presently considering a project, to follow on the Plant Sites Red Data Book, that would attempt to predict where the most plant extinctions are occurring and will occur if present trends continue. This will link together data on the distribution of plant diversity with data on loss of habitats. The project is still at an early stage and funding has not yet been obtained.

Biogeographical approaches have also been suggested as the best 'short cut' to deciding which areas to protect in the time available. The Nature Conservancy's impressive programme in Latin America is based on this theme as is in part the related IUCN project to assess the coverage of protected areas worldwide (described by MacKinnon and outlined in 3.3 of Thorsell's paper). The difficulty is that the more tropical an environment becomes, in general the less effective are biogeographical techniques for conserving plant species. In other words, a set of protected areas chosen on biogeographical criteria may protect most species of, say, Arctic Canada, even if only covering a tiny part of the land surface, but would not protect most species of the Peruvian Andes, where plant distributions do not reflect biogeographic boundaries, many species being confined to far smaller areas. A.H. Gentry, for example, has found that some Andean valleys have very different floristic composition to neighbouring valleys of similar environment (pers. comm.).

The answer is surely that no one approach provides a single answer. A judicious mixture is needed. In many of the most acute cases, as in the Atlantic forests of Brazil, common sense and local knowledge may be the best immediate answer, as the policy should simply be to protect what little is left. Here the detailed species lists and biogeographical studies are important, but for long-term planning and management rather than for immediate rescue action.

4. THE USE OF INFORMATION TECHNIQUES FOR PLANT CONSERVATION AND FUTURE APPROACHES

There is a large and rapidly expanding literature of Red Data Books, national threatened plant lists and other reports on plant conservation, although mainly for the temperate and subtropical countries. Like much conservation information, much of it is 'grey literature', that is reports, often a mimeographical typescript, produced in low numbers and known only to a handful of people. Such reports are usually freely available but cannot be said to be effectively published.

To provide a ready reference to this obscure but burgeoning literature, the IUCN Conservation Monitoring Centre (CMC) is publishing a book entitled

Plants in Danger: What do we know? (Davis et al., in press). Arranged by country and island, it outlines data sources on plant conservation and answers questions such as "Where can I find out about the flora of a country? Which of the plants are known to be threatened? Who then can I get in touch with?"

In particular the country accounts in the book give detailed references of all reports and publications known to IUCN that list and describe threatened flora. Brief analyses show the number of threatened and extinct species wherever known. To put this information into context, the accounts contain short notes on the flora and vegetation as a whole; in virtually all cases these include the number of native plants, a unique feature. To assist the working conservationist, the book provides references to the most up-to-date Floras, Checklists and Field-Guides, as well as listing the major Botanic Gardens and voluntary organisations concerned with plant conservation in each country.

The CMC have built a database for most of the threatened plant lists and national Red Data Books covered in the book. At present this principally covers rare and threatened species and is essentially a matrix of:

(a) Plant name (in Latin, with authorities);
(b) Areas in which that plant occurs (using a set of c. 565) codes covering all countries, states in federal countries (e.g. U.S.A., Mexico) and most individual islands or island groups;
(c) IUCN Red Data categories for the degree of threat, applied at area and world level, where known;
(d) Various other descriptors.

The contents of the database are presently as follows:

Number of plants	35,303
Number of plants rare and threatened worldwide	15,907
Number of plant-area records	44,274

The 15,907 rare and threatened plants are made up as follows:

Extinct	342
Endangered	2646
Vulnerable	2425
Rare	4977
Indeterminate	5517

(30 December 1985)

Detailed data-sheets, comprising one or more sheets of text, are held on c. 300 of the threatened plants, including those published as The IUCN Plant Red Data Book (Lucas and Synge, 1978). Research for the Plants in Danger book has shown that there are a number of threatened plant lists not incorporated into the database. It is reasonable to assume, therefore, that the total number of known threatened plants will rise to maybe as high as 20,000 taxa during 1986 as these data are incorporated.

In future this should be extended to include also:

1. Extinction-prone species (i.e. species in high-risk areas but not assignable to Red Data Book categories at present.)
2. The major economic species (from the list to be developed, as described in 3.2).

The database reflects the sharp discrepancy observed with national Red Data Books and floristic richness. This is illustrated in Table 7.

TABLE 7

Comparison of floristic richness with number of taxa in IUCN database

Region	No. Species[1]	No. Threatened Species in Database[1]
Europe	11,500	2282 (20%)
North America	20,000 (?)	2171 (11%)
Africa (with Madagascar)	30,000	1942 (7%)
Central America	?	1620
South America	80,000	116 [2]

1. Taxa of vascular plants; all world-threatened except Europe, which are regionally-threatened.
2. Temperate South American threatened plant lists not yet screened for inclusion in the database; add say at most 500 taxa.

The development of the database is accompanied by a vigorous policy of decentralisation. In the 1970s, for Europe at least, the threatened plant list prepared by IUCN pre-dated most national lists and stimulated the production of many of them. When the first version of this list was published in 1977, there were few national lists available; however, when the second appeared in 1983, there was a flurry of activity at national level, so much so that a third version is planned for the late 1980s to incorporate all the additional data now available. The best approach is for each country to produce their own threatened plant list and for this material to be fed into the international database. The IUCN database is increasingly becoming an overview of information held nationally, with pointers to where that data may be found. The role of an international body like IUCN is seen as to provide methodology, to provide technical aid, and to fill the gaps, rather than undertake the compiling of such information itself.

This process of decentralisation has been greatly helped by the decreasing cost of computer power. Many countries want to develop databases, either specifically on threatened plants or, more often, on the environment in general and so including plant data. In the coming years IUCN intends to put emphasis on promoting national databases, and under a current project partly funded by the European Research Councils, IUCN will prepare a Database Blueprint to outline the minimal informational content essential for a national, general-purpose database for plant conservation. IUCN, and hopefully other organisations, will use this as a means of encouraging national institutions to set up national plant conservation databases and promoting compatibility and exchange of information between existing databases.

The Blueprint will open the way to the development of a far more coherent and effective network of national databases for plant conservation. During 1986, IUCN will be considering how best to develop and promote such a network of national databases on plant conservation and the necessary links between them. A vital ingredient will be to set up a forum, where all involved can meet regularly and work collectively on problems of data collection, software development and data compatibility. Funds, however, for this work are presently lacking; of the order of US$50,000 - 100,000 per year would probably be necessary to get the project started; further money may be needed for hardware purchase and staff time in each country involved, although in some cases this could be covered locally and by bodies like the World Wildlife Fund. (The present thinking is to use IBM PC ATs, costing around US$10,000 each, but later on adding the GIS software and hardware that has just become available on this machine, at an extra US$30,000.)

At present IUCN and the leading Botanic Garden in the Canary Islands are making plans to develop a database for plant conservation in those islands, which will serve as a model for other national databases. Botanists and plant conservationists from many other countries have expressed interest in national databases on plants and a number have asked IUCN for help to set them up. In particular, there is considerable interest in Mauritius, India, Nepal, Hong Kong, to name a few areas of priority for plant conservation. The Nature Conservancy, a U.S. NGO, is managing an impressive programme of institution-building around the development of conservation databases in Latin American countries.

The problem of compatibility is a complex and difficult one, not limited to the botanical field. As every researcher gets a desk-top computer, each is tempted to design a database for their own application. Yet so often, each application is similar, though not identical, to that of a fellow scientist in the same discipline. One lesson, now very clear, is that each scientist or institution has to go through a slow learning curve in this new and exciting process. Home-made databases, later abandoned, are an essential part of this process. The institutional difficulties in getting agreement on common standards are formidable, but there are encouraging signs that they can be overcome. On the one hand, IUCN received endorsement from representatives of around 200 leading Botanic Gardens to continue development of an International Transfer Format for records of plants growing in Botanic Gardens - this would enable easy transfer of data from one garden to another, eventually perhaps to form an international, shared database on which species are in which Botanic Gardens. Secondly, the leading plant taxonomic institutions of the world, notably Geneva, Kew, Missouri, New York, have joined together to form a Taxonomic Databases Working Group; this met for the first time in Autumn 1985 and agreed an ambitious work programme for the following year or so. Only time will tell whether these initiatives will succeed or whether in five years or so there are literally thousands of over-lapping but incompatible databases around the world on related topics.

In parallel with the work on the promotion and development of national databases, IUCN plan a major development of the CMC database for 1985-6, incorporating the main features to be outlined in the Database Blueprint (see above). These includes distribution by localities, including presence in named protected areas, synonyms, life-form, bibliographic references and data sources. It will include continued research on effective coding schemes for threats and for habitats. Work to do this, however, is only partly funded and the CMC computer needs replacement with a more up-to-date model that can handle GIS data. Additional funding, both capital and regular, is needed for CMC to be effective as a public service to the world community on the provision of conservation data.

How accurate are the data? It is hard to say. In many countries, like Spain and Australia, the national data are the result of workshops and study groups that involve most or all the botanists in the country. Here we can be confident that the assessments are more or less correct, especially if the list or book is in the second or third revision. In other countries, however, the work is the result of the work of one scientist. However gifted he or she may be, the likelihood of error is greater. Assessing a species for degree of threat and its chances of survival is a subjective matter and depends on judgement; the more people who have an input to the assessment the more likely it is to be correct.

The best way to confirm the accuracy of a list of threatened plants is to prepare more detailed data on each species and so validate the assessments made. This has been slow to happen, partly because of the vast number of species involved, but is likely to speed up if national databases on plant conservation are created, as outlined above. It is essential in its own right as identifying a species to be threatened is clearly only the beginning of efforts for the conservation of that species. There are many other elements of information that are needed. The most critical of these are data on precisely where the plant occurs - its present localities. Parallel to this are data on population biology - how many plants occur at each locality, what are the bottlenecks in the life-cycle to expansion of the population, and so on. The techniques to do this are fairly sophisticated, following for the most part J.L. Harper's work on population biology (Harper, 1977). Good examples of such studies are few, some being given in a conference partly devoted to this theme (Synge, 1981); this shows rather clearly that the techniques evailable are as diverse as the number of experimenters!

A useful guide for data-gatherers is the guidelines developed by Henifin and colleagues (1981) for the preparation of status reports on rare or endangered plant species, designed for the requirements of the U.S. Endangered Species Act of 1973. The very structured approach may be suitable for some experimenters, and provides a useful checklist of factors to consider.

Experience to date shows how important individual studies are before rescue attempts are undertaken. In a number of cases, the fate of the plant has been harmed by well-meaning but incorrect conservation action. This is especially true for small, very vulnerable sites, where mistakes can be fatal. A good example is the story of the Ranunculus ophioglossifolius in Britain, outlined by Frost (1981). Early efforts to conserve the principal population, confined to a site of 1/12 acre, eliminated the plants altogether, as the fence erected by the conservationists kept out the grazing animals which maintained the habitat. Fortunately in this case, the seed remaining in the soil was sufficient for the plant to regenerate when the habitat was grazed again. The tragedy is that for those regions where most plants are threatened, conservation of individual plants has never been attempted, so there are no stories of success or failure to recount. This is all the more serious because of the large number of economic species in the tropics.

There has been little systematic assessment of which species, especially rare and threatened ones, are in existing protected areas. IUCN is keen to include data of this kind in the CMC database, since one part of CMC is a database on the world's protected areas, with over 9,000 entries so far. Yet in most cases knowledge on rarity in plants comes from taxonomists. Few park managers have a list of the plants in the sites they manage and those lists that do exist are often difficult to obtain and unreliable. Unesco, with IUCN's help, is very interested in sponsoring a database within each of the designated Biosphere Reserves; if this develops into an effective program, it may provide the momentum to assemble the data required.

Indeed, in the coming decades, as habitats continue to decline, I see emphasis moving away from identifying threatened species towards cataloguing the occurrence of all species in protected areas. One can then ask the question, Which species are not protected at all?, and give priority to them. This is a much less subjective approach. The difficulties, however, are formidable: to derive the list of species not protected, one needs first of all an agreed list or database of plants of the region concerned, and then that all park lists follow that list. Such agreement in taxonomy is still far off, but modern computer technology should act as a spur for regional and specialist plant databases, linked together in a network, perhaps even a world plant database.

In contrast, the data are far better on which of the threatened species are conserved *ex situ* in Botanic Gardens, although it is generally accepted that this approach is a second-best solution and is unlikely to succeed for most tropical plants. IUCN's Botanic Gardens Conservation Co-ordinating Body link together c. 250 Botanic Gardens into a world network for plant conservation and will be greatly expanded following the recommendations of an international conference on Botanic Gardens and the World Conservation Strategy (Las Palmas, November 1985). Surveys have shown that c. 4000 of the c. 16,000 known threatened species are recorded in cultivation in Botanic Gardens. This total is likely to rapidly expand, double even, as exchange of electronic media becomes possible between Botanic Gardens and IUCN using the International Transfer Format (see above). Although the data are good, cultivation in a garden is not the best means of conservation: living collections in Botanic Gardens are vulnerable to loss, and frequently cover only a tiny proportion of a plant species' genetic variation. The great challenge here is to develop a seed bank network, learning from the lessons of the FAO/IBPGR network for major crops, that will provide effective *ex situ* conservation for all species that need it. This will be a target for the expanded version of the Botanic Gardens Conservation Coordinating Body to be set up later in 1986 and will doubtless require substancial funding.

5. INFORMATION ON THE DECLINE OF PLANT SPECIES IN THE UNITED STATES

by R.A. DeFilipps

The most recent comprehensive listings of plant species, subspecies and varieties, whose survival may be imperiled on a national basis, are contained in the *Federal Register* (U.S. Fish and Wildlife Service, 1980, 1983, 1985). The lists were prepared from data solicited, researched, and collated by botanists of the Office of Endangered Species, U.S. Fish and Wildlife Service, Department of the Interior, Washington, D.C. The species are arranged in the federal documents as follows: Taxa Currently Listed (as officially Endangered, or Threatened); Taxa Currently Under Review; Taxa No Longer Under Review.

The lists in the Federal Register ultimately derive from the Smithsonian Institution report on recommended plants prepared in response to a U.S. Congressional directive in the Endangered Species Act of 1973, entitled Report on Endangered and Threatened Plant Species of the United States. An update of the Smithsonian lists, with a list for Puerto Rico and the Virgin Islands, was published as Ayensu and DeFilipps (1978). This publication, still in print, lists 839 "endangered", 1211 "threatened" and 90 "extinct" taxa for the continental United States (including Alaska). The figures when combined represent approximately 10-11% of the mainland U.S. flora (which totals c. 20,000 species). During the interval between the 1975 and 1978 Smithsonian lists, several species presumed extinct were rediscovered due to the interest generated by the lists, the most notable being the Furbish lousewort (Pedicularis furbishiae) of Maine and the Virginia roundleaf birch (Betula uber).

The Smithsonian lists have no legal standing. The official government list of Endangered and Threatened Wildlife and Plants is published by the U.S. Fish and Wildlife Service, most recently in a 27 September 1985 version. The lists include common name of the species, scientific name, historic geographical range and status (endangered, or threatened).

Since listing (and, as well, de-listing) is a continuing process, the established method for the public to keep aware of the Service's actions is to consult the proposals and final rulemakings published by law in the Federal Register. Seeing this as a handicap, the Service also publishes the easily obtainable Endangered Species Technical Bulletin, each month. The tables in the October 1985 issue show that to date there have been officially listed 82 endangered plants and 23 threatened plants (species occurring only in the U.S.). Prior to this issue, the Bulletin of January 1985 listed the 33 species for which approved Recovery Plans, for restoring listed plant populations to a self-sustaining level, have been developed.

It has long been realised that the lack of modern published Flora for the United States as a whole has impeded the inventorying of certain data required to study plant species population dynamics and distribution in places such as the remoter parts of the southeastern U.S. Individual states, however, often have published Floras and most have developed either officially sanctioned state-appointed committees or voluntary groups and advisory committees to deal with problems of endangered plants at state and local levels. Since the Endangered Species Act of 1973 does not prohibit the picking of endangered plants, and applies only to nationally significant plants growing on federally administered land, such state-sponsored groups have a vital role. Virtually each of the 50 states is represented by a state of rare, endangered, and threatened plants or species of concern; they have been most recently surveyed and appraised in an article by Ayensu (1981), which is brought up to date in Plants in Danger : What do we know? (referred to above). General knowledge of larger regions is bulked up by the appearance of publications such as Kral (1983) and Nelson and Arndt (1981). Two additional publications may be singled out as excellent expositions of a national and regional perspective on the endangered plant species issue: the proceedings of a New England Symposium (Anon, 1980), and Morse and Henifin (1981).

Due to constraints of political and economic importance, some officials continue to acknowledge only reluctantly the conservationists' vision of a state emergency inherent in plant decline and loss. In Hawaii, the need for funds generated by timber and agricultural harvests and urban development has often been influential enough to seemingly overpower the eagerness of local conservationists to apprehend the invasion and deterioration of native plant cover by exterior forces. Yet, for Hawaii, ample evidence of the fragile nature of the ecosystems and the 1,100 endangered, threatened, and extinct taxa has been expounded by various authors in numerous articles and no fewer than three books by Professor Sherwin Carlquist of the Rancho Santa Ana Botanical Garden in California: Carlquist (1965, 1974 and 1980).

As pointed out by Senator Lee Metcalf and Congressman Keith Sebelius (The Nature Conservancy News (28(1): 6-12, 1978), "In 1975 the President's Council on Environmental Quality estimated that 1.25 million acres of natural and agricultural land - an area the size of Delaware - are annually converted to more intensive uses. Urban expansion alone consumes 740,000 acres; transportation projects 130,000 acres; and reservoirs 30,000 acres". In fact, a county map of the United States showing loss in natural vegetation types in each county (Klopatek, 1979) shows that among the types that have already lost more than 50 per cent of their potential area are the Florida Everglades, the California steppe, the Southern USA flood-plain forest, bluestem grass prairie, and the beech-maple forests of north-central United States.

Files of endangered plant species information are maintained by the U.S. Fish and Wildlife Service - Office of Endangered Species as background information and solicited testimony and data contracted from collaborators around the country. The Plant Conservation Unit of the Department of Botany, Smithsonian Institution, maintains data previously used to corroborate the 1975 and 1978 listings of species recommended for protection pursuant to the Endangered Species Act.

A determined effort to maintain and expand data files on endangered species has been made consistently over the years by The Nature Conservancy (Arlington, Virginia), a private organisation which has developed a sophisticated "element-based inventory" system by means of which to categorize the biological factors and biota occurring in a given area, as an aid to natural areas selection. It is perhaps the only active national-oriented data gathering system to be seriously reckoned with at the present time, and its services are in constant and growing demand.

The most desired overall situation, yet to be achieved, would be the presence of a published Flora of the United States; the adoption of a stronger political determination to include plant conservation factors in plans to develop the natural environment to human requirements within the states; the further encouragement of a system of national data resources for endangered plants; the encouragement of a larger output of officially listed and protected species; more state laws protecting specific plant species; and the continual education of a public for some of whom the Endangered Species Act of 1973 will always be a potential adversary.

6. CONCLUSIONS

6.1. Our generation is living through - and is causing - the first mass extinction event in the plant kingdom. The most threatened floras include those of mediterranean ecosystems, of islands and of tropical forests.

6.2. The decline in diversity is obvious, but not readily quantified. One particular difficulty is the lack of knowledge on tropical floras, where plant diversity is greatest and threats to vegetation are most acute. Two thirds of plant diversity is tropical but most botanists live and work in the temperate countries. Thus the greatest study has gone into the areas of least diversity. Shortage of tropical botanists skilled in tropical plants is now a critical factor hampering plant conservation.

6.3. So far 15,907 higher plant taxa have been categorised as rare or threatened but this omits many tropical floras where endangerment is high. At least 60,000 plant species may be at risk of extinction within the next 30-40 years. Yet it will not be possible to identify many of these species until they have become extinct.

6.4. Therefore alternative approaches are needed in addition to the conventional identification of threatened plants. One of the most promising is to identify those key sites richest in plant diversity and then to promote their protection; the best - perhaps the only - way to conserve most tropical plants will be to protect large areas of intact vegetation.

6.5. Approaches to identify habitats in most danger are also useful but are difficult to apply and constrained by lack of vegetation maps and biogeographical analyses of floras. In the tropics, especially the wet tropics, no set of protected areas chosen solely to represent each habitat or vegetation type or biogeographic unit could contain all the plant species of the region.

6.6. Documentation on plant conservation worldwide, with present emphasis on threatened species, is held in the plants database of the IUCN Conservation Monitoring Centre. This database is emerging as an overview of data at national level, to which it contributes and which it promotes. With adequate funding, it could be instrumental in the development of a network of national plant databases. Computer technology has been useful in managing information on plant diversity, but compatibility problems constrain optimal use.

6.7. The best data are on economic species, but mostly on the c. 100 or so crops covered by the IBPGR system, notably in the ex situ gene banks. There is a major vacuum on the conservation status and exact distributions of the tens of thousands of other economic plants, especially medicinal species, timber and firewood trees (though covered to some extent by FAO), and ornamental species; and a subsequent lack of in situ conservation action both for these species and the major crops.

6.8. Simply knowing which species are rare or threatened or prone to extinction is not enough to plan their conservation. Detailed data are required, e.g. on localities, habitat, population biology. A critical gap is lack of plant lists for most protected areas in the tropics - without these there is no easy way to assess which species are protected so far.

6.9. Data are better on ex situ conservation, which is seen as a necessary adjunct to in situ conservation. About one third of recorded threatened plants are conserved ex situ, mainly in Botanic Garden collections. The priority is to develop a seed bank network in parallel to the Botanic Garden collections.

6.10 For the United States, the lack of a national Flora is a critical and embarrassing gap. So far c. 2100 species have been demonstrated to be rare or threatened, yet the Endangered Species Act covers little more than 100 of them and the desperate situation facing much of the flora seems not to be fully appreciated by policy-makers. In particular, far more attention should be given to covering the plant wealth of Hawaii, much of which is on the verge of extinction.

7. RECOMMENDATIONS

Plant conservation has perhaps lagged behind other parts of conservation in that no action plan has been prepared specifically to address the needs of plants. The structure for such a plan exists in the IUCN/WWF Plants Conservation Programme (described by Synge, 1984); this is a set of activities designed by IUCN for funding by WWF from the funds generated by their Plants Campaign. (Its intention is to make plant conservation accepted as a fundamental part of conservation as a whole. About half of the US$3 million promised has been committed so far and the programme is well on schedule.) The most pertinent set of general proposals made is that prepared by the IUCN/WWF Plant Advisory Group at their first meeting in 1984; this is appended as Annex 2 and should be read in conjunction with the rest of this section.

This OTA paper shows clearly the mismatch between the extent of loss of plant diversity and the available knowledge with which to combat it. The following actions are recommended:

7.1. The creation and management of a worldwide network of protected areas for _in situ_ conservation of biological diversity. Conservationists have argued that this should cover about 10 per cent of the earth's surface; the proportion of plant diversity covered by such a network will probably depend more on where the areas are situated than the total amount of land protected.

Governments should give far more technical aid and support for the creation of more large protected areas in the tropics and the consolidation of existing ones. Many bodies are already active in this field, especially WWF and the Unesco World Heritage Convention, and many government aid agencies are becoming so, but far more is needed. Details on how this may be done are outlined by J. thorsell in his paper for OTA on protected areas in the tropics, especially section 7.5.1.

As mentioned above, IUCN is preparing an indicative inventory of c. 150 sites at present under threat but which if conserved could save the most plants. This is surely the most cost-effective means of plant conservation. IUCN estimate that, assuming that the governments involved wish to conserve the sites but lack the resources to do so, a reasonable assumption, aid of US$100 - 200 m will be needed over 5-10 years. Since the book by definition contains the sites where most plant extinctions could be prevented, its implementation would be the single most cost-effective means of plant conservation.

Another target for aid should be the making of inventories for the plants occurring in tropical protected areas.

7.2. The present emphasis on threatened species needs to be continued in the developed countries, but the emphasis moved from data-gathering to action. Although more data is needed, and will always be needed, there is enough available for action to be taken. It is a matter of much regret that of the c. 2000 species threatened in the continental United States (excluding Hawaii), only 78 have been formally listed under the provisions of the Endangered Species Act, now 12 years old. And it is important to note that the Hawaiian Islands hold more recorded endangered and extinct plants than any other island on earth and more than any single country also. More emphasis should be given to a programme of biological conservation in the Hawaiian islands, including both _in situ_ and _ex situ_ methods, the latter to be spearheaded by the Botanic Gardens there.

7.3. Botanists and plant conservationists should put far more emphasis on economic plants. For too long we have given equal emphasis to each species, rather than concentrated on the useful ones. Ways also have to be found to put the burden of cost for economic plants onto the consumer.

It is well known that the genetic resources of the major crops used in the United States, and in the rest of the northern hemisphere, occur predominantly in developing nations. While the U.S. has greatly supported the efforts by IBPGR to build an _ex-situ_ network of seed banks for the conservation of land-races of crops, very little has been done for the _in situ_ conservation of the wild relatives of those crops. This new field should be a major focus for technical aid.

Further and more precise recommendations on economic plants will doubtless be included in the economic plant papers in this series.

7.4. Efforts should be made to reinforce national and international databases on plants and their conservation. At international level, financial support is needed for the IUCN Conservation Monitoring Centre to reach its full potential as an overview of data on conservation worldwide. The CMC database, which covers plants (as outlined above), animals, protected areas, wildlife trade and some ecosystems, has many applications to the work of U.S. institutions, especially US-AID, the State Department and the U.S. Department of the Interior, as well as international agencies based in Washington.

At national level, a programme of support for national databases is recommended, using common standards to be adopted through appropriate bodies, like IUCN. The first requirement is to build a consensus on how such a project could be devised.

7.5. Governments should provide far greater support for plant taxonomy as the basic discipline upon which conservation of plant diversity relies. This support should have several components - funding for training in plant taxonomy for students from many countries; the creation of far more permanent posts in the subject in the universities and institutions of the United States; and the development of national and international electronic databases of taxonomic information that can make information on plant diversity and distributions easily available to a multitude of users. Raven and Crosby (in a parallel paper) estimate that six times more taxonomists are needed than presently assigned to provide adequate data to plan tropical plant conservation before it is too late; this would cost about US$ 500 m per annum.

7.6. The launch of a major consciousness-raising exercise to bring the biological diversity issue to the attention of policy-makers and the public at large. Part of this would be a clearer understanding of how policies in one nation - in this case the United States - affect biological diversity in another. The hamburger connection has been widely paraded, and the effect of developed world timber imports upon tropical rain forest is well known, but there may be other, similarly important issues that have not yet been aired.

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ANNEX 1

DEFINITIONS OF THE IUCN RED DATA CATEGORIES

Extinct (Ex)

Taxa which are no longer known to exist in the wild after repeated searches of their type localities and other known or likely places.

Endangered (E)

Taxa in danger of extinction and whose survival is unlikely if the causal factors continue operating.

Included are taxa whose numbers have been reduced to a critical level or whose habitats have been so drastically reduced that they are deemed to be in immediate danger of extinction.

Vulnerable (V)

Taxa believed likely to move into the Endangered category in the near future if the causal factors continue operating.

Included are taxa of which most or all the populations are decreasing because of over-exploitation, extensive destruction of habitat or other environmental disturbance; taxa with populations that have been seriously depleted and whose ultimate security is not yet assured; and taxa with populations that are still abundant but are under threat from serious adverse factors throughout their range.

Rare (R)

Taxa with small world populations that are not at present Endangered or Vulnerable, but are at risk.

These taxa are usually localized within restricted geographical areas or habitats or are thinly scattered over a more extensive range.

Indeterminate (I)

Taxa known to be Extinct, Endangered, Vulnerable or Rare but where there is not enough information to say which of the four categories is appropriate.

Out of danger (O)

Taxa formerly included in one of the above categories, but which are now considered relatively secure because effective conservation measures have been taken or the previous threat to their survival has been removed.

In practice, Endangered and Vulnerable categories may include, temporarily, taxa whose populations are beginning to recover as a result of remedial action, but whose recovery is insufficient to justify their transfer to another category.

Insufficiently known (K)

Taxa that are suspected but not definitely known to belong to any of the above categories, because of the lack of information.

N.B. For species which are neither rare nor threatened, the symbol 'nt' is used.

ANNEX 2

CONCLUSIONS OF IUCN/WWF PLANT ADVISORY GROUP MEETING
St Louis, Missouri
17-18 December 1984

Plants are a primary resource of fundamental importance for human life. Rapid population growth, together with the excessive and increasing demands that are placed on the world's resources by our societies, are threatening to destroy a major portion of our common heritage of plants. This threat is especially evident in the tropics and subtropics, where at least two-thirds of the plants of the world occur, and where the process of deforestation is proceeding at an alarming rate. Widespread poverty, famine, and political instability, for example in Africa, are manifestations of the same processes that are driving plants to extinction and, by doing so, seriously limiting our future options for developing sustainable relationships between man and his living resources.

All human beings depend upon plants, directly or indirectly, for their lives, as do most other forms of life: at least four million different kinds of organisms depend on about 250,000 kinds of plants. But unless we immediately begin to take drastic and innovative measures to preserve them, it is likely that tens of thousands of plant species will disappear forever during our lives or those of our children. Their loss would amount to a fundamental and permanent change in the character of life on earth, a life whose wealth is characterized by great diversity.

Some 20 plants provide more than 85% of our food and only a few hundred species are cultivated widely. Most plant species have never been examined to see if they might have properties that would make them useful as food or for other purposes in our modern industrial age, and thousands of species have not even been given a name or described scientifically. It is against this background that an international committee of botanists, meeting at the Missouri Botanical Garden in St. Louis on 17-18 December 1984 at the request of World Wildlife Fund (WWF) and the International Union for the Conservation of Nature and Natural Resources (IUCN), reached the following conclusions:

1. Tropical Plants. At least two-thirds of the plants in the world occur in tropical and subtropical countries. They constitute the least known set of plants on earth. At the same time, they comprise, together with endemic island floras, the most highly endangered plants on earth. The potential for discovering useful new plants is greater in the tropics than elsewhere, but many tropical plants are being lost before they are properly understood. For these reasons, special attention should be paid to the tropical and subtropical plants.

2. Inventories. Urgent attention should be given to completing surveys of plant diversity and distribution in many areas of the world, especially the tropics and subtropics. Without this basic information, the rational use of plant resources cannot be undertaken. This inventory work is required to provide the foundation for other efforts in the area of plant conservation.

3. Protected Areas. Our knowledge of the protected areas of the world is inadequate for the best management of these areas for the long-term preservation of genetic diversity. Surveys of UNESCO Biosphere Reserves and similar areas should be initiated as a way of uniting the biological and conservation communities and expediting the world inventory of the living resources contained within protected areas.

4. Reserves. When natural and semi-natural areas are converted to man-made ecosystems, a minimum of 10% of the land should be established as reserves for plant genetic resources. These areas should include as much of the natural diversity of the region as possible, especially because of the general lack of knowledge about individual plant species that would allow informed decisions to be made. Adequate inventories of the sort outlined above would provide a rational basis for such planning before the actual development of the areas.

5. Economic Plants. Populations of economically important plant species should be preserved either by in situ or by ex situ methods, or both, as appropriate.

6. Unique Plants. Biological distinctiveness, as well as economic potential, should be emphasized in selecting particular plant species for special conservation measures.

7. Land-use Planning. Botanists should be included in land-use planning teams, particularly in tropical regions where the available knowledge is especially limited. Land-use specialists such as agronomists and foresters should be included routinely in conservation-oriented discussions. Conservationists must also work more actively with agriculturists and foresters, bearing in mind that conservation and sustainable development can succeed properly only if they go hand in hand.

8. Man-made Landscapes. Many varieties and races of economically important plants occur only in man-made landscapes; these races often can be preserved effectively only by preserving areas where they are routinely maintained. For areas that have already been cleared, as much consideration as feasible should be given to reforesting them with native species and to the restoration of biological diversity.

9. Traditional Uses of Plants. Increasing scientific knowledge of traditional plant uses and preserving such uses are important goals since such knowledge is rapidly disappearing in the face of the loss of traditional cultures resulting from many of the trends mentioned above.

10. Collaboration. Only active collaboration among institutions of all kinds can lead to the improvement of the present situation, to world stability, and to the rational utilization of the plant resources on which all of our lives depend. The unequal distribution of economic wealth and species diversity makes collaboration between developed and developing countries essential, but a mugh higher degree of collaboration than now exists among developing countries is likewise of great importance.

11. Dialogue between Botanists and other Conservationists. Greater efforts should be made both on the part of the traditional conservation community and on the part of the community of botanists to interact more extensively so as to be able to work together efficiently for the preservation of the diversity of plants on earth.

12. Conservation Information. Those dissiminating conservation information should pay special attention not only to its distribution in developed countries, but also, and much more extensively than at present, to those who live in developing countries. This information should be written in the appropriate language, and should utilize the most suitable media.

13. Botanical Gardens. Botanical gardens can provide the most important single point of information for the public on plant conservation issues, and they should be strengthened and encouraged to play this critical rôle more effectively.

14. Planning for Botanical Gardens. An overall strategy should be developed for botanical gardens so that they are better able to fulfil their missions in conservation research and education. This strategy would involve the creation of a worldwide network of botanical gardens, improved training opportunities for botanical garden staff, and enhanced research into the conservation of threatened and endangered plant species in botanical gardens.

15. Training for Conservation. Effective action in conservation demands well-trained scientists and technicians, but there is a severe shortage of such people especially in the tropics. Specifically, the proper management of germplasm reserves differs greatly from park management in general, and is seldom based on adequate information. To address this important problem, increased efforts need to be made to provide appropriate opportunities for training at all levels, to incorporate conservation principles as a normal part of biological and botanical training, and to encourage the preparation of outstanding textbooks and other curricular materials pertinent to this field. The establishment of specific degrees in conservation might profitably be extended to universities beyond those where such degrees are offered at present.

Contract Paper No. 2

Status and Trends of Agricultural Crop Species

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Office of Technology Assessment
U.S. Congress

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ABSTRACT

This paper is a review and assessment of the data available on the current status and trends of genetic diversity of agricultural crops. The paper covers the 30 leading crops in the US (average annual value of farm sales + imports combined).

The genetic diversity of a crop comprises the variety of genes in the crop itself (traditional and modern cultivars [cvs]) and in those wild relatives from which gene transfer is practicable. Genetic diversity is maintained at three levels of security: long term maintenance (50-150+ years); short term maintenance (up to 50 years); and no provision for maintenance (wild populations outside protected areas, and currently grown cvs). This paper is primarily concerned with the data on status and trends of crop genetic diversity at this third level, since here the risk of outright loss is greatest.

Current data on status and trends of crop diversity are published in the Plant Genetic Resources Newsletter (FAO & IBPGR), IBPGR reports, crop specialist newsletters and (to a limited extent) in scientific journals. The data are usually presented in the form of brief impressionistic statements on cv loss, threats to wild species, and similar instances of genetic erosion. Quantitative data are seldom provided--quantitative data on trends being particularly lacking--and there is generally little or no basis for comparing reports on genetic change in a particular crop or region with data on the status and trends of other crops or other regions.

Nonetheless the data appear to be adequate for two of the three purposes for which they are needed: (1) to stimulate conservation action (chiefly collection of germplasm for ex situ storage); (2) determination of general collecting priorities among crops and among regions. Several hundred collecting missions have collected 100,000 samples in 88 countries under the auspices of IBPGR alone, and in the decade 1974-1984 the number of genebanks with long term storage facilities increased from eight to 55. These and collecting missions organized by other international and national agencies have been and continue to be planned according to priorities determined in part (sometimes a minor part) by current data on status and trends in crop genetic diversity.

However, the data are inadequate for the development of a more broadly based strategy of genetic conservation--one that complements long term ex situ storage with in situ protection of wild relatives and maintenance of old and local cvs by associations of farmers and gardeners. Development of such a strategy requires quantitative data on changes in cv composition and in wild populations, combined with information on the cultural, economic and ecological factors that influence those changes. Quantitative data are also needed to plan and carry out more specific and localized collecting missions. Rice is used to illustrate the need for better socio-economic data on cv change.

Better data are needed for four main groups of crops: (1) low international (IBPGR) priority crops of major economic importance to the US (such as grape, sunflower, forage crops, ornamental crops); (2) crops maintained in living collections (such as cacao, rubber, sugarcane, Citrus, banana); (3) crops whose collection and characterization are well advanced (such as rice, maize, tomato); (4) wild relatives of major crops (such as those listed above).

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PART 2 (APPENDIX). SUMMARY OF DATA ON STATUS AND TRENDS OF GENETIC DIVERSITY OF SELECTED MAJOR CROPS (EXCLUDING TIMBER CROPS) 2.1

REFERENCES R1

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This paper is a review and assessment of the data available on the current status and trends of genetic diversity of agricultural crops. The paper is organized into two parts. Part 1 contains the review and assessment proper. It covers the 30 crops (excluding timber crops) that contribute most to the US economy, each having a total annual farm sales plus import (customs) value in 1980 of $300 million or more (soybean to onion in Table 1). Together, the top 30 crops make up 60% of the US plant resource base (the combined annual value of all plant resources, wild and domesticated, produced and imported by the US) (Prescott-Allen & Prescott-Allen. In press.) Part 2 (Appendix) provides a summary of data on the status and trends of genetic diversity of a representative sample of these crops (the 13 crops in the families Alliaceae-Gramineae), to illustrate the scope and form of the available data.

PART 1. REVIEW AND ASSESSMENT

By genetic diversity is meant the variety of genes in a crop. These are found in the crop itself and in related wild and domesticated species. Using the tripartite classification of Harlan & de Wet (1971), the crop and its relatives form a series of up to three gene pools, defined according to the ease of crossability and gene transfer from the related taxon to the crop. The crop and its closest relatives (those with which it is interfertile and from which gene transfer is generally easy) constitute the crop's primary gene pool (GP1), domesticated forms being designated GP1A and spontaneous forms GP1B. The secondary gene pool (GP2) includes species from which genes can be transferred by conventional means despite crossing barriers and high (but not complete) hybrid sterility. The tertiary gene pool (GP3) is made up of species that produce completely sterile hybrids when crossed with the crop but from which gene transfer is possible using radical techniques (such as embryo culture, colchicine treatment, bridging species). (Further definition of the three gene pools is provided in explanatory note 4 of Part 2 [page 2.1].)

The genetic resources of the crop itself (the GP1A) can be classified into three categories:

- Landraces or traditional cultivars
- Modern cultivars (subcategorized into cultivars in current use and obsolete cultivars)
- Special genetic stocks (including induced mutations)

Table 1. Crops grown or imported by the USA with a combined annual value of $100 million or more (average 1976-1980)

AVERAGE ANNUAL VALUE OF CROP US FARM SALES & IMPORTS (US$ MILLIONS)	CROP	FAMILY
11,278	Soybean	LEGUMINOSAE
10,412	Corn	GRAMINEAE
6,475	Wheat	GRAMINEAE
4,233	Cotton	MALVACEAE
3,925	Coffee	RUBIACEAE
2,851	Tobacco	SOLANACEAE
1,723	Sugarcane	GRAMINEAE
1,525	Grape	VITIDACEAE
1,206	Potato	SOLANACEAE
1,163	Rice	GRAMINEAE
1,150	Sweet orange	RUTACEAE
1,147	Sorghum	GRAMINEAE
1,054	Alfalfa	LEGUMINOSAE
1,051	Tomato	SOLANACEAE
1,016	Cacao	STERCULIACEAE
815	Apple	ROSACEAE
760	Beet crops	CHENOPODIACEAE
747	Peanut	LEGUMINOSAE
706	Rubber	EUPHORBIACEAE
672	Barley	GRAMINEAE
527	Lettuce	COMPOSITAE
517	Common bean	LEGUMINOSAE
393	Sunflower	COMPOSITAE
368	Banana	MUSACEAE
365	Cole crops	CRUCIFERAE
355	Almond	ROSACEAE
349	Peach	ROSACEAE
314	Coconut	PALMAE
304	Oats	GRAMINEAE
287	Onion	ALLIACEAE
252	Strawberry	ROSACEAE
219	Grapefruit	RUTACEAE
198	Chrysanthemum	COMPOSITAE
192	Cucumber	CUCURBITACEAE
189	Melon	CUCURBITACEAE
186	Pineapple	BROMELIACEAE
179	Roses	ROSACEAE
167	Celery	UMBELLIFERAE
164	Walnut	JUGLANDACEAE
158	Peppers/chilis	SOLANACEAE
156	Jute	TILIACEAE
155	Plum/prune	ROSACEAE
148	Sweet cherry/sour cherry	ROSACEAE
146	Pear	ROSACEAE
144	Olive	OLEACEAE
143	Oil palm	PALMAE
142	Carrot	UMBELLIFERAE
142	Pea	LEGUMINOSAE
136	Lemon	RUTACEAE
130	Bermudagrass	GRAMINEAE
128	Tea	THEACEAE
116	Watermelon	CUCURBITACEAE
116	Cashew	ANACARDIACEAE
110	Sweet potato	CONVOLVULACEAE
102	Pecan	JUGLANDACEAE
100	Azalea/rhododendron	ERICACEAE

Source: Prescott-Allen & Prescott-Allen. In Press.

The neutral terms "traditional" and "modern" are preferable to their commonly used counterparts, "primitive" and "advanced". The genetic diversity of traditional cvs has two dimensions: between sites and populations; and within sites and populations. The former is mainly a matter of spatial heterogeneity; the latter a matter of both spatial and temporal heterogeneity (change between seasons and in response to longer term ecological and socioeconomic change) (Frankel & Soule 1981). As modern cvs have replaced traditional cvs both dimensions of genetic diversity have declined: fewer cvs are grown and the cvs are more uniform (but see also the paragraph on the sequence of varietal change, page 1.13).

This decline in diversity has generated two distinct concerns. First, concern over the extinction of genes, genotypes and gene pools (populations), resulting in the outright loss of the raw materials of crop improvement. Second, concern that increased uniformity of crops increases their vulnerability to destruction by pests and diseases. The second concern is the lesser because: (a) uniformity is reversible while genetic extinction is not; (b) it is the availability of alleles for resistance, not genetic diversity *per se*, that reduces a crop's vulnerability to pests and pathogens (Brown 1983); (c) the uniformity of a crop can be reduced and resistance alleles can be introduced as long as a high degree of genetic diversity overall is maintained (in collections and protected areas as well as in growers' fields) and is available for use by breeders. For several major crops in the United States, breeders keep ready for use when needed a substantial supply of genetic diversity in the form of cultivars under trial, parental lines, synthetic populations and other breeders' stocks (Duvick 1984). In other words, the second concern is a dependent concern, the first is basic.

This is not to belittle concern over uniformity, particularly over cytoplasmic uniformity, whether due to wide use of the same maternal parent as in modern cvs of rice (Hargrove, Coffman & Cabanilla 1979) or to use of a single source of cytoplasmic male sterility as in sunflower hybrids (90% of the crop: Fick 1978) and sugarbeet hybrids (Bosemark 1979). The emergence of a pathotype that is virulent on a widely used cytoplasm could destroy a high proportion of the crop (as did race T of *Helminthosporium* maydis with respect to T cytoplasm maize [Committee on Genetic Vulnerability of Major Crops 1972]). Point (c) above still applies, however: as long as alternative cytoplasms are identified, conserved and incorporated in breeding lines (and as long as the crop is monitored for outbreak of new pathotypes) the crop can be protected.

The total genetic diversity of a crop is maintained at three levels of security: (1) long term maintenance (50-150+ years), as provided by long term seedstores and in vitro collections and by protected areas (such as nature reserves); (2) short term maintenance (up to 50 years) and specialist use, as provided by breeders' and research collections and by grass-roots (associations of farmers and gardeners) collections of traditional and local cvs; (3) no provision for maintenance (including general current use), as exemplified by wild populations outside protected areas and by modern and traditional cvs currently grown by farmers and gardeners (other than specialist collectors). These three levels and their components are linked by gene flow, with germplasm transferred from level 3 to levels 2 and 1 returned to level 3 by the introduction of new cvs. The ideals of conservation are: (a) to keep as much of the total genetic diversity as possible somewhere in this system; (b) to have as much as possible at level 1, where the risk of outright loss is least; (c) to have as much as possible at the other levels as a matter of insurance. Because of the limited storage capacity of level 1, redundancy of accessions maintained at that level is generally undesirable; but redundancy among the three levels adds to the security of the system.

This paper is primarily concerned with data on the status and trends of genetic diversity at level 3 (wild populations in their native habitats and currently grown cvs, particularly traditional cvs), since here the risk of outright loss is greatest.

Data available on the status and trends of crop genetic diversity

The first and probably the last attempt at a comprehensive survey of the status and trends of crop genetic diversity was organized in the early 1970s by the Food and Agriculture Organization of the United Nations (FAO) and the International Biological Programme of the International

Council of Scientific Unions (IBP) (Frankel 1973). The survey concentrated on traditional cvs of food crops in their centres of diversity with the aim of directing germplasm collection efforts to those areas where genetic diversity was still great and where the risk of its loss was high. Nonfood crops were considered a lower priority and wild and weedy relatives were regarded as being less immediately threatened with extinction. Because of the need to assemble the information quickly, before the genetic resources reported on disappeared, the survey relied entirely on the field notes and other informal reports of scientists working in the regions, plant explorers, evolutionists, crop specialists and the like (Frankel & Soule 1981).

The informal reports of scientists and others who work most closely with crop genetic diversity (plant breeders, taxonomists, geneticists, economic botanists) continue to be the main source of data on status and trends of crop diversity. The data are published in the *Plant Genetic Resources Newsletter* (of FAO and the International Board for Plant Genetic Resources [IBPGR]), the reports of IBPGR (notably its series of reports on the genetic resources of particular crops), reports of germplasm collection expeditions (for example, Berding & Koike 1980), and the crop specialist newsletters (such as the *Cruciferae Newsletter*, *Cucurbit Genetics Cooperative*, *Oat Newsletter*, *ISSCT Sugarcane Breeders' Newsletter*, *International Pigeonpea Newsletter*). The crop specialist newsletters vary greatly in the apparent interest of their correspondents in the status and trends of the genetic diversity with which they work (reports often appearing in some of them--for example, the *Cruciferae Newsletter*--and seldom or not at all in others). *Economic Botany* covers the issue, and scattered data can be found in other scientific journals but at a very low frequency. For wild relatives of crops, additional sources of data are the Red Data Books and other national and regional lists of rare and threatened plant species described by Synge (1985) in another paper in this series.

The scope and form of the available data are illustrated in Part 2 (Appendix), which either quotes directly from the original sources or summarizes them in language that is close. In general, there is at least some information on the GP1A of each crop, and the amount and detail of the information declines from GP1 to GP3. Exceptions are those crops (such as sugarcane) in which substantial breeding effort and germplasm collection is devoted to the GP2 and GP3, with a corresponding increase in information on the status of the genetic diversity of those gene pools. The form of the data is usually a brief, impressionistic statement: "it may not be long before landraces are irretrievably lost"; "many locally adapted cvs have been replaced by modern cvs"; "in several countries important old cvs have been lost"; "the risk of losing significant amounts of germplasm is already high and is expected to increase"; "Beta maritima is in danager of genetic erosion". Some statements are extremely brief: genetic erosion in a country is stated as being "high" or "low" or a species is classified as "rare", "endangered" or "vulnerable" without any supporting data. Information on status that is quantified is exceptional; quantified information on trends is more exceptional still.

Quality of the available data

The quality of the available data is best assessed in light of the purposes for which they are gathered. The two main purposes have been to stimulate conservation action (chiefly collection of germplasm for ex situ storage) and to help in determining priorities within and among crops. A third purpose is seldom stated but might reasonably be assumed: to help in developing effective conservation strategies (that is, appropriate mixtures of institutional ex situ, grass-roots ex situ, and in situ conservation).

With respect to the first purpose the data have been entirely adequate. Since the early 1960s when FAO and IBP began to mobilize concern about genetic erosion, and particularly since 1974 when IBPGR was established, the global state of germplasm collection and storage has been transformed. Between 1974 and 1984 IBPGR fielded more than 300 collecting missions, which collected more than 100,000 samples in 88 countries (Lawrence 1984). During this period the number of genebanks with longterm storage facilities increased from eight to 55 (Hansen, Williams & Freund 1984; Plucknett et al. 1983). IBPGR has concluded that by 1990 all major cereals and several other crops will have been adequately collected. From 1984 it has shifted its operational emphasis from general collecting over wide areas to a combination of more specific and detailed collecting on the basis of intensive fieldwork (ecogeographical surveys), accelerated collection of wild materials

(GP1B, GP2, and in some cases GP3), and post-collection activities (characterization, documentation, multiplication, and duplication of samples in genebanks) (IBPGR 1984; Lawrence 1984).

The adequacy of the data for determining priorities among and within crops is more difficult to assess. Since 1976 IBPGR and the many national and international agencies with which it cooperates have determined priorities among crops and regions and organized exploration and collection accordingly. The status and trend of genetic diversity of each crop has been only one of the criteria used, others being the economic and social importance of the crop, the requirements of plant breeders and research workers, and the size, scope and quality of existing collections (CGIAR 1976). This last criterion, which should be rather easy to apply, has instead been very difficult, due to inadequate characterization and documentation of accessions and considerable redundancy in the collections (for example, about 60% of the 85,000 accessions of barley in European collections are duplicates) (IBPGR 1984). In recent years it has been supplemented with a related one--progress in implementing action plans for each crop, particularly progress with collection initiated in the 1970s and 1980s.

By the end of 1983 IBPGR had developed action plans and begun their implementation for all global first priority crops (wheat, Phaseolus beans, cassava, sweet potato, coffee, tomato) and for 85% of the global second priority crops (IBPGR 1984). As an example of the effectiveness of these action plans, 12 of the 25 countries of the Mediterranean, Southwest Asian and Central Asian regions designated for germplasm collection under the wheat action plan were thought to have been adequately collected by the end of 1983 (IBPGR 1984). The action plans vary in the importance attached to genetic erosion as a criterion for determining priorities. Of 12 recent action plans, six base their priorities 50/50 on genetic erosion and on some other criterion (usually representation in existing collections, but also economic importance and breeding needs): onion (Astley, Innes & van der Meer 1982); cole crops and other crucifers (IBPGR 1981b); sugarcane (IBPGR 1982b); coffee (IBPGR 1980); tomato (Esquinas-Alcazar 1981); and cacao (IBPGR 1981d). The other six do not explicitly relate the priorities to genetic erosion (although it is usually mentioned in general discussion) or treat it as a minor criterion: sweet potato (IBPGR 1981a); wheat (IBPGR 1984); Phaseolus beans (IBPGR 1984); banana (IBPGR 1978b); coconut (IBPGR (1978a); and Capsicum crops (peppers/chilis) (IBPGR 1983).

On the face of it, therefore, the data have been adequate for their second purpose, since priorities among and within crops have been determined and acted on. As noted in the last section (page 1.21), several crops of major economic importance to the US are undergoing genetic erosion and their long term conservation is poorly provided for--but it is doubtful that better quality data on genetic erosion alone would have changed their status. There is no question that during the 1960s and 1970s traditional cvs of many crops were being lost at such a rate that the need for rapid and concerted collection greatly outweighed that for precise data. Partly because many landraces have disappeared from farms and gardens, and partly because much of the germplasm collection most urgently needed has been done, conservation requirements have changed. Collecting is still needed but it should be targetted more precisely. The longterm maintenance (including regeneration) of the germplasm collected so far must be assured; and the germplasm should be evaluated. Finally there is a need for a broader strategy of germplasm conservation--one that makes use of all means of conservation rather than being dependent, virtually exclusively, on ex situ storage by government agencies and major research institutions.

Both the development of such a strategy and the devising of more specific collecting work demand better quality data. Currently data are most precise for areas where genetic erosion is complete or almost complete and for wild relatives with restricted distributions (narrow endemics). Examples of the former are: the winter onion crop in Egypt now consisting of a single cv (page 2.3); the complete replacement of local cvs of wheat by introduced cvs in the Caspian Sea zone of Iran (page 2.22); and the abandonment of spelt as a crop in the Netherlands together with the loss of the last remaining collection of Dutch landraces (page 2.24). Examples of the latter are: the halving of known surviving populations of Helianthus paradoxus from four to two (page 2.6); the halving of the range of wild annual taxa in the GP1B of maize (page 2.30); and, on the positive side, confirmation of the low risk of genetic erosion of Beta procumbens and B. webbiana (page 2.5), Helianthus anomalus (page 2.6), and Brassica cretica (page 2.9).

The next level of precision is illustrated by the data on rice in South and East Asia (pages 2.15-2.17), which indicate the percentage of the crop area still grown to traditional cvs. Unfortunately, there is no indication of what relationship (if any) there is between the area occupied by traditional cvs and the number and diversity of the cvs still being grown. Reports that combine data on area and on numbers of cvs are rare and difficult to interpret. In a survey of Central Luzon (Philippines) the proportion of the wet season rice area grown to traditional cvs dropped from 100% in 1966 to 33% in 1970, 32% in 1974, and 6% in 1979; the number of traditional cvs grown dropped from 25 in 1966 to 11 in 1970 and 1974, and 8 in 1979 (Cordova et al. 1981). This suggests that the rate of cv loss was not as great as the decline in cv area. However, lists of cv names can give a false impression of diversity, since several names may be given to essentially the same landrace or (conversely) the same general term may be applied to several different local cvs. In some cases (for example, wheat in mountain areas of Greece, page 2.25), the apparent persistence of traditional cvs may mask the replacement of highly heterogeneous populations by a few, less diverse landraces.

In fact the better data raise as many questions as they answer and point up the need for detailed analyses of those factors that accelerate genetic erosion and those that promote grass-roots conservation of genetic diversity (loyalty to traditional and other local cvs).

While there have been a number of studies on the diffusion and adoption of modern cvs, apparently little attention has been paid to the persistence of traditional cvs (which may not simply be the obverse of modern cv adoption). There are great differences in the rate and extent of adoption of modern cvs--among crops, among countries, and among different parts of the same country. These differences have generally been attributed to environmental factors. Since their almost simultaneous introduction on a large scale in the mid-1960s, modern (short stature, fertilizer responsive, photoperiod insensitive) cvs of wheat have come to occupy a larger proportion of the wheat area than have modern rice cvs of the rice area (Table 2). The difference has been explained by the greater size of the total rice area and its greater environmental heterogeneity, so that a much greater diversity of types of rice cv is needed to provide the same biological advantage over local cvs (Herdt 1980).

Table 2. Estimated area of modern cvs of wheat and rice in developing countries, 1976/77.

Region	----Wheat----		----Rice----	
	000 ha	% of wheat area	000 ha	% of rice area
South & East Asia	19,672	72	24,200	30
West Asia & North Africa	4,400	17	40	4
Tropical Africa	225	23	115	3
Latin America	5,100	41	920	13
TOTAL	29,397	44	25,275	28

Note: data rounded to nearest 1,000 ha & 1% (500 ha & 0.5% rounded up)
Source: Dalrymple 1978

The rather narrow range of environments to which modern rice cvs have been adapted (until recently) help to explain differences in the extent of their adoption among the countries of South and East Asia (Table 3). The modern cvs have been almost completely adopted in the fully irrigated, more fertile parts of the region. These are areas where farmers' risks are relatively low, predisposing them to invest in the fertilizer, pesticides and water control needed to realize the high yield potential of the new cvs. By contrast the bulk of farmers growing rice under rainfed conditions have not adopted the modern cvs because of the higher risks involved and the high cost, impracticability or unavailability of the technologies required to obtain reliable yields. (Jennings, Coffman & Kauffman 1979). Thus in Indonesia modern cvs are largely confined to irrigated and rainfed lowland below 500m elevation (72% of the rice area). Above 500m the modern cvs take a longer time to mature and have high levels of sterility, and traditional cvs are preferred (Bernsten, Siwi & Beachell 1981).

Table 3. Area of modern cvs of rice in South and East Asia, 1978/79.

Country	000 ha	% of rice area
Bangladesh	1,768	18
Burma	288	6
India	16,881	42
Indonesia	5,216	63
Korea (Rep. of)	929	76
Malaysia	316 (1977/78)	53 (1977/78)
Nepal	313	25
Pakistan	1,015	50
Philippines	2,512	72
Sri Lanka	491-611*	68-85*
Thailand	1,100	12

Notes: data rounded to nearest 1,000 ha & 1% (500 ha & 0.5% rounded up)

*depending on definition of modern cv (higher figure includes "H" cvs developed in Sri Lanka & introduced in the late 1950s & early 1960s)

Source: Herdt 1981

In Sri Lanka the "new improved" cvs (short stature) are grown in the so-called stable rice environments with adequate water, fertile soils and high solar radiation. "Old improved" cvs (the "H" cvs, developed from landrace material) are grown in the "moderately stable" rice environments where water supply is less assured and soils are less fertile. The traditional cvs remain in pockets of "unstable" or poor rice environments with mineral excesses or deficiencies and subject to flooding, drought and other problems (Gunawardena 1980). In Bangladesh modern cvs occupy 62% of the boro rice (irrigated dry season) area (1978/79 data) but only 13% of the aus rice (rainfed dryland) area and 12% of the aman rice area (transplanted aman rice is rainfed, broadcast aman is deepwater) (Herdt 1981). The modern cvs are quite unsuitable for vast areas of Thailand because of lack of irrigation water or of water control (Awakul 1980).

Many other factors are involved, of course. The rapid diffusion of the modern cvs in Indonesia has been attributed to a combination of rehabilitation and expansion of the irrigation infrastructure, organization of farmers' groups, effective extension services, government pricing policies (for chemical inputs as well as for the crop) and the incidence of brown planthopper damage (to which PB32 [Indonesia IR32] and its successor cvs are resistant) (Bernsten, Siwi & Beachell 1981).

Jennings, Coffman & Kauffman (1979) suggest that the ecological and social factors that limit the adoption of improved cvs (and hence foster the persistence of traditional cvs) are strongest in a crop's centre of origin. They believe that four generalizations can be made about the improvement of major food and industrial crops within their centres of origin:

> "Yields are lowest within the centers of crop origin.
>
> "Yield-limiting factors are most numerous and complex in the centers of crop origin.
>
> "Resistance to changes in crop production methods is greatest in the centers of origin.
>
> "The impact of any technology is least within the centers of crop origin."

Among the factors that explain these characterizations, Jennings and his colleagues cite two. First, that within their centres of origin crops are subjected to intense pressure from pathogens and pests that have co-evolved with them ("the number of important pests within areas of origin always exceed that found in distant production areas"). Second, that crop varieties that have developed through natural selection in their centres of origin are "vegetatively large and vigorous and have great intra- and inter-specific competitive ability". Improvement of their yield potential inevitably reduces that competitive ability.

These generalizations may apply to regions of diversity rather than centres of origin, and more to tropical regions of diversity than to temperate or Mediterranean ones. Even if they are only partly applicable, they have profound implications for conservation strategy, since they suggest that in some regions of diversity there may be a limit to genetic erosion. The recent history of varietal change in rice lends support to this suggestion. Varietal change has generally been in the sequence: traditional cvs - phase 1 modern cvs (pureline selections of landraces; improved cvs of conventional height) - phase 2 modern cvs (high response, broadly adapted cvs; improved cvs of short stature) - phase 3 modern cvs (high response cvs adapted to local conditions). The first two phases involve a progressive decline in genetic diversity; but the third seems likely to increase genetic diversity (of the crop in regions where the cvs are grown) through the incorporation of diverse sources of germplasm (including local and other landrace material) adapted to the various local agroecosystems. In India, for example, improvement through phase 2 modern cvs greatly increased yields on some 25% of the rice area; but development of high response cvs suitable for the remaining 75% (dryland or poorly drained areas) has required programmes to breed location-specific cvs (Pande & Seetharaman 1980). Most of the donors for resistance to major biotic and abiotic stresses have been Indian, such as the traditional deepwater cv Nagaribao crossed with the phase 2 modern cv Pankaj (IR5) to obtain high response deepwater lines (Pushkaran & Rao 1985).

Examination of the Jennings-Coffman-Kauffman hypothesis, and of its relevance to trends in crop genetic diversity, requires more detailed and precise data on the cvs themselves (genetic and agronomic characterization of each cv), on the actual changes in their use (time series data), and on the ecological, cultural and economic variables that influence their use. Cultural and economic data are as important as ecological data, it being unlikely that traditional cvs survive only in those agroecosystems that are hostile to the modern cvs. Java-Bali, the largest area of modern cvs in Indonesia, is also the largest area of traditional cvs (Table 4). A strong motive for growing them is that several (for example, the scented cv Rojolele) command a higher price than modern cvs. After years when brown planthopper damage was not significant, farmers have tended to revert to these cvs (Bernsten, Siwi & Beachell 1981).

Table 4. Average area of traditional cvs of rice in Indonesia, wet seasons 1977/78 to 1979/80

Region	000 ha	% of rice area
Java-Bali	680	18
Sumatra	470	32
Kalimantan	410	60
Sulawesi	120	24
Nusatenggara & Timor	90	32
TOTAL	1,770	27

Source: Bernsten, Siwi & Beachell 1981

Farmers' taste preference for "high quality rice" is also given as one of the main reasons for the persistence of traditional cvs in north Sumatra (Bernsten, Siwi & Beachell 1981). Scented or aromatic traditional rices (such as Basmati of India and Pakistan, Dulha Bhog of Bangladesh, Khao Dawk Mali of Thailand, Azucena and Milfor of the Philippines, and Rojolele of Indonesia) are prestigious and expensive (Khush, Paule & de la Cruz 1978). Among the 1302 old and recent local cvs collected in Japan from 1962 to 1965, 42 were old scented cvs (mostly from Kyushu in the south). Perhaps more striking still is that some 45% of the cvs were glutinous, probably because breeding effort has concentrated on nonglutinous cvs and because the glutinous cvs were grown almost exclusively for home consumption and so "farmers were indifferent to the exchange of varieties" (Nagamatsu & Omura 1975). Glutinous or waxy rices are grown in Japan and China for sweet confections and other special dishes and are the staple rice in Laos and northern Thailand. They are characterized by an almost complete absence of the starch amylose.

Most rices are nonglutinous (or nonwaxy): the japonica cvs of temperate regions have low (10-20%) amylose and cook moist and sticky; javanica cvs of Java, Bali and the hill terraces of Banaue (Philippines) have intermediate (20-25%) amylose and cook moist and tender, remaining tender when cool; typical indica cvs of the Indian subcontinent, Burma, southern Thailand, Vietnam and most of China have high (25-30%) amylose content and cook dry and fluffy, becoming hard on cooling (Awakul 1980; Bernsten, Siwi & Beachell 1981; Khush, Paule & de la Cruz 1978). A survey by the International Rice Research Institute (IRRI) has found that even where indica cvs are grown intermediate amylose rices are preferred (Basmati, for example, is intermediate) (Khush, Paule & de la Cruz 1978). However, all but four of the 23 cvs released by IRRI between 1966 and 1980 have high amylose (Khush pers comm. 1982). It is not surprising that traditional cvs persist in situations in which a premium is put on taste and cooking quality. (Grain quality involves other variables besides amylose content--length, shape, appearance, proportion of whole grains recovered from milling, time required for cooking, tenderness, aroma--which add to the complexity of breeding rices with high consumer acceptability.)

Similar examples can be found for other crops and other regions. Villagers along the eastern slopes of the Peruvian Andes prefer traditional cvs of potato to modern cvs because they can grow the former without the expense of buying seed, fertilizer and pesticides and because only the traditional cvs have the texture and taste they expect (Brush 1977). There may in fact be fairly well defined circumstances in which the special qualities of local cvs outweigh the modern cvs' advantages of high yield and high return per unit of labour (even in areas where the risks of using the modern cvs are low). With respect to food crops it is possible that genetic erosion will be highest in that portion of the crop grown for sale, while persistence of local cvs will be greatest in that portion of the crop grown for home consumption. It is a hypothesis worth exploring.

An understanding of the cultural and economic factors that may promote the loss of some categories of cv and the persistence of others and that may lead some individuals and communities to adhere to their local cvs while others discard them is essential for more effective conservation of crop genetic diversity. In developed countries a valuable additional means of conserving germplasm has emerged with the rise of organizations of gardeners and farmers dedicated to maintaining and exchanging old and local cvs. Examples are Seed Savers Exchange and Native Seeds/SEARCH in the US, Canadian Organic Growers in Canada, and Henry Doubleday Research Association in Britain. Similar initiatives could be started in developing countries, perhaps using the very organizations that have helped to diffuse the modern cvs. An example is Subak, the indigenous water users' association in Bali (Indonesia). Subak is a highly organized social system that has been mobilized for the introduction of new technology, and to which many observers have attributed the rapid introduction and adoption of the new rice cvs (Bernsten, Siwi & Beachell 1981). In Sri Lanka the Henry Doubleday Research Association has set up a "sanctuary for rice and vegetable varieties" in cooperation with the Sri Lanka Rural Organization (Hills pers. comm. 1983). Grass-roots conservation efforts are well worth encouraging because they combine and reinforce individual loyalties to traditional cvs and constitute an additional line of defence against genetic erosion.

The data available on the status and trends of genetic diversity of the wild relatives of crops are just as vague as those on the crop themselves but rather fewer. As noted on page 1.8, they are most precise for species with restricted distributions. Red Data Books and lists of threatened plant species are concerned with extinctions of species (and sometimes subspecies), not with losses at the population level, so they tend not to treat species that are widespread. Although the plant breeding and other crop specialist literature contains references to genetic erosion of wide-ranging wild species, quantitative data are practically nonexistent. The report on populations of the spontanea form of rice in one county in China (page 2.18) is exceptional.

Unsupported statements about the lack or insignificance of genetic erosion in wide-ranging wild relatives are occasionally made but should be viewed with scepticism, since they may be influenced by invalid assumptions of the species' resilience and uniformity. Many wild relatives are adapted to disturbed, unstable habitats, flourishing as weeds in the expanding home provided them by people; so their ubiquity would seem assured. However, they differ greatly in the types of disturbance and degree of instability to which they are adapted: some weeds are aggressive, readily taking advantage of newly disturbed areas; others are meek, tolerating only a rather narrow range of disturbance. The arundinaceum race of wild sorghum is an example of the former type, while the aethiopicum race is an example of the latter (page 2.22). Many species have weed populations in one part of their range and populations that are components of the primary vegetation in another part (*Hordeum spontaneum*, diploid *Medicago sativa*, and *Lycopersicon pimpinellifolium* are examples). Many are also highly variable both within and among populations. High allelic endemism (alleles occurring at a frequency of 5% or more in only one population) has been found in autogamous species such as *Hordeum spontaneum* (Nevo *et al*. 1979) and *Lycopersicon cheesmanii* (Rick & Fobes 1975), allogamous species such as the GP1B relatives of maize (Smith, Goodman & Stuber 1984) and *Lycopersicon pennellii* (Rick & Tanksley 1981), and species that are both such as *Lycopersicon pimpinellifolium* (Rick, Fobes & Holle 1977). These last authors observe that plant breeders have tended to underestimate the variability of wild species, testing small numbers of accessions from limited portions of the species' range, apparently assuming that any gene is fixed in the entire species. Sampling the extremely small collections of wild relatives in *ex situ* storage would tend to support this assumption, since the sample would be unlikely to reflect the variability of the species. This in turn would reinforce the view that the small collections are representative and that depletion and loss of wild populations does not entail significant genetic erosion.

General conclusions

The general conclusions that can be drawn are limited by the nature of the available data: variable and incomplete coverage and scant quantification. There is clearly a consensus among plant breeders, plant explorers and other germplasm collectors that genetic erosion has occurred and is still occurring rapidly and widely in many crops. What are the worst affected areas and crops and what are the detailed relationships between social and economic changes and changes in crop genetic diversity cannot be determined. What may be "serious genetic erosion" for one commentator may not be for another.

Are the status and trends of genetic diversity of some crops better reported than those of others? Table 5 presents an assessment of the coverage and quality of the data on the 30 crops reviewed for this paper. Separate assessments are shown for the different gene pools of each crop to see whether data on the GP1A are better than those on the other gene pools. Some published sources of data may well have been overlooked, but even allowing for this there appear to be clear differences in coverage, though not in quality, from crop to crop. In general, crops that are high IBPGR priorities (W1, W2, R1) are more comprehensively reported than lower priority (W3, W4) and no priority crops. This may reflect differences among crop specialists in use of IBPGR media rather than differences in knowledge. Quantitative data are published on few of the crops; and reports that quantify cv replacement directly (rather than state changes in area grown to a general class of cvs) seem to be confined to potato, specifically to reports by Ochoa. The GP1As of the crops are more comprehensively reported than the other GPs, except when the other GPs include other crops (for example, the GP2 of rice, GP2 of common bean, and GP3 of cole crops) or when the GP1B is smaller than the GP1A (for example, cotton and cacao).

Constraints on acquiring an improved understanding of the status and trends of crop genetic diversity

A better understanding of the status and trends of crop genetic diversity requires first of all a precise description of the extent and nature of that diversity. This in turn requires: (1) morpho-agronomic (and preferably also isozymic) characterization of cvs and wild relatives (including the major variants of the wild relatives) in the GP1B, GP2 and (if likely to be used) GP3; (2) knowledge of the phylogeny or evolutionary history of the crop. Only when the variability and degree of differentiation of the crop (GP1-GP2/3) has been determined can losses (or gains) in genetic diversity be assessed. The point is obvious but easily overlooked. A further requirement is the gathering of time series data: for example, an area that has been explored in detail (and collections from which have been adequately characterized) could be revisited and collected again. A final requirement is an account of the cultural, economic and ecological factors that influence the observed changes in domesticated and wild populations of the crop.

Table 5. Coverage and quality of data on status and trends of genetic diversity of 30 crops of major economic importance to the USA

CROP	IBPGR PRIORITY	---DATA ASSESSMENT---				
		GP1A	GP1B	GP2	GP3	
1. Soybean	W2 R1	C/S	C/S	---	P/Se	
2. Maize	W3 R1	P/Sq	C/Sq	P/Se	nd	Appendix
3. Wheat	W1	C/Sq	---	C/Sq	P/S	Appendix
4. Cotton	W2	P/S	C/S	P/S	Fe	
5. Coffee	W1	P/S	C/S	nd	nd	
6. Tobacco	---	P/S	---	nd	nd	
7. Sugarcane	W2 R1	C/S	C/S	P/S	incl. in GP2	Appendix
8. Grape	W3	F	P/S	nd	ni	
9. Potato	W2	C/SQ*	F*	P/SQ*	F*	
10. Rice	W2 R1	C/Sq	C/Sq	C/S	F	Appendix
11. Sweet orange	W2 R1	P/S^{+}	P/S^{+}	nd^{+}	P/Se^{+}	
12. Sorghum	W2 R1	P/S	P/S	---	nd	Appendix
13. Alfalfa	---	F	F	F	F	
14. Tomato	W1	C/S	P/S	P/S	P/S	
15. Cacao	W2 R1	C/S	C/S	nd	nd	
16. Apple	W2 R1	P/S	F	ni	ni	
17. Beet crops	W2 R1	P/S	P/S	nd	P/Se	Appendix
18. Peanut	W2 R1	F	nd	nd	nd	
19. Rubber	W2	C/S	F	incl. in GP1B	ni	Appendix
20. Barley	W2 R1	C/S	nd	---	nd	Appendix
21. Lettuce	W4	F	nd	nd	nd	Appendix
22. Common bean	W1	P/S	nd	P/S	P/S	
23. Sunflower	W3	F	F	P/SQe	nd	Appendix
24. Banana	W2 R1	P/S	C/S	P/S	P/S	
25. Cole crops	W2 R1	P/S	P/S	---	P/S	Appendix
26. Almond	W4	P/S	F	ni	ni	
27. Peach	W2	P/S	F	ni	ni	
28. Coconut	W2 R1	F	F	---	---	
29. Oats	W3	F	F	F	P/S	Appendix
30. Onion	W2 R1	C/S	nd	P/Se	nd	Appendix

Key to Table 5

IBPGR PRIORITY = IBPGR priority rating (IBPGR 1981c)

W1, W2, W3, W4 = global first, second, third, fourth priority

R1 = regional first priority

--- = not rated by IBPGR

DATA ASSESSMENT = assessment of published data with respect to

coverage: C = comprehensive (most of the countries of occurrence, though not necessarily in detail or in all parts of each country)

P = partial (some/a few countries or species)

F = fragmentary (one country or species, or general statements made without reference to a particular country or species)

quality: S = standard (unquantified reports)

q = quantified but indirect reports (extent or rate of genetic erosion not directly stated but may be inferred; e.g. "area grown to traditional cvs has declined by 20%)

Q = quantified reports that directly express status or trends of genetic diversity (e.g. "distinct local types declined from 25 to 20")

e = data on endemic species only

--- = none (no such gene pool)

nd = no data (no published data seen, though some may exist)

ni = not identified (gene pool not identified for purposes of this paper)

GP1A, GP1B, GP2, GP3 = primary A, primary B, secondary, tertiary gene pools (defined on pages 1.1 and 2.1)

* Potato GP1A *Solanum tuberosum*; GP1B wild tetraploids in Series Tuberosa; GP2 remaining Tuberosa spp + Series Conicibaccata, Demissa, Longipedicellata, Acaulia, Commersoniana, Cuneolata, Megistacroloba, Piurana (some spp in these Series may be GP3); GP3 remaining spp in *Solanum* Section Petota.

+ Sweet orange GP1A here includes all domesticated *Citrus* spp; GP1B all wild *Citrus* spp; GP2 *Fortunella* and *Poncirus* spp; GP3 *Clymenia*, *Eremocitrus* and *Microcitrus* spp.

Appendix = data on this crop are summarized in Appendix (Part 2)

This statement of requirements suggests the main constraints on acquiring an improved understanding of the status and trends of crop genetic diversity. They are time, money, and the institutional problems of sustaining multidisciplinary research. As observed earlier, however, an improved understanding of the subject is an essential aid to more specific collecting, more broadly based conservation, and the development of more effective ways of combining the benefits of crop improvement with maintenance of the genetic materials of crop improvement.

Major gaps and areas of needed study

The gaps fall into four main groups:

1. Low international (IBPGR) priority crops of major economic importance to the US. Chief among these are tobacco, grape, alfalfa, lettuce, sunflower and oats. At least three of these species (grape, alfalfa and sunflower) are either insufficiently collected, or lack adequate provision for long term conservation, or both. There are few large collections of sunflower species and only one (the N.I. Vavilov All-Union Research Institute of Plant Industry, USSR) is capable of long term maintenance. The largest collection of wild *Helianthus* species in North America (the USDA collection at Bushland, Texas) is a working collection. Organized exploration, collection and maintenance of the wild species in particular is considered a high priority (Beard 1981; Dedio & Putt 1980; Fick 1978). Grape is another crop whose wild component (widely and increasingly used in breeding) is undergoing genetic erosion, although which species and areas are worst affected is not known. *Ex situ* collections of *Vitis* species (other than *V. vinifera*) are deficient, poorly representing both inter- and intra-specific diversity (McGuire & Qualset 1984, Mortensen 1974). Many collections occupy areas that are too small and face problems of maintenance; much valuable germplasm has already been lost (Mortensen 1974). Alfalfa requires systematic collection as a matter of priority, as well as an improved system of germplasm maintenance (Barnes *et al*. 1977). Forage species in general lack adequate conservation. Ornamentals form another category of crop of great economic importance whose conservation has been neglected. For

example, there is no germplasm collection of chrysanthemum (McGuire & Qualset 1984) -- the 33rd most valuable crop in the US (Table 1: page 1.2).

2. Certain crops that despite being a high international priority still lack adequate provision for long term conservation. These include species with recalcitrant seeds (cacao, rubber, coconut) which at present must be maintained in living collections, as well as several crops with orthodox seeds that nevertheless are maintained as living plants (coffee, sugarcane, Citrus, banana). The maintenance capacity of field genebanks (living collections) is obviously much more limited than that of seedstores. This limitation puts a premium on accurate determination of the cultivated and wild populations most in need of conservation.

3. Crops whose collection and characterization are well advanced, notably rice, maize, potato, tomato and common bean (Phaseolus vulgaris). These crops would be excellent candidates for detailed assessments of the status and trends of genetic diversity of the GP1A (+ the GP1B and possibly GP2 of maize, and all GPs of tomato). The aim should be studies, along the lines described in the previous section, of the crops in a region of important and well described diversity (for example, ne India for rice; Mexico and/or Peru for maize, potato, tomato and common bean). Such studies are not the luxury they might appear. Not only are they needed to advance understanding of the dynamics of crop genetic change and its relationship to social and economic change; they are also needed to refine remaining collection work (still "far from complete" for tomato [McGuire & Qualset 1984], for example) and to direct repeat collection of populations of maize (for example) that may have been lost as a result of melodramatic storage problems (Timothy & Goodman 1979).

4. Wild relatives of major crops. For most crops (sugarcane and tomato are exceptions), wild relatives are represented in collections by extremely small samples. To assess the risks of genetic erosion, larger samples representative of the ecological variation of the GP1B, GP2 (and for some crops the GP3) should first be collected and characterized.

Assessment is possible already for the limited number of wild relatives whose variation (in morpho-agronomic characters, isozymes, or other traits such as disease resistance) has been reasonably well described. They include: *Helianthus annuus*, *Avena sterilis*, *Hordeum spontaneum*, *Triticum turgidum dicoccoides*, wild *Zea* spp. and ssp, *Lycopersicon cheesmanii*, *L. pimpinellifolium*, and *L. pennellii*.

PART 2 (APPENDIX). SUMMARY OF DATA ON STATUS AND TRENDS OF GENETIC DIVERSITY OF SELECTED MAJOR CROPS (EXCLUDING TIMBER CROPS)

Explanatory notes

1. Major crops are defined here as crops making a major contribution to the US economy, having a combined annual farm sales plus import (customs) value of $300 million or more.

2. Crops are listed in alphabetical order by family and by scientific name within family. Genera are assigned to families according to Willis (1980). Six families are covered: Alliaceae, Chenopodiaceae, Compositae, Cruciferae, Euphorbiaceae and Gramineae.

3. The combined annual farm sales plus import value of each crop is indicated alongside the crop's name. Unless stated otherwise values are for 1980. US farm sales data are from USDA (1982); US import data from US Department of Commerce (1981). Values are shown in millions of US dollars. The figures in parentheses following the value indicate the percentage accounted for by farm sales and imports respectively. Example: $350m (92/8) -- 92% of the $350m comes from farm sales, 8% from imports.

4. Data on status and trends of genetic diversity of each crop are summarized according to the gene pool classification of Harlan & de Wet (1971), as modified by Smartt (1984):

   GP1: primary gene pool, corresponding to the traditional concept of the biological species, and consisting of --

   GP1A: domesticated forms of the crop (landraces and bred cultivars);

   GP1B: wild taxa (including weed forms) that are interfertile with the crop and hybridize readily with it.

GP2: secondary gene pool, consisting of those biological species that can be crossed with the crop using conventional breeding methods to produce at least some fertile progeny. GP2 is equivalent to the coenospecies. Gene transfer from GP2 to GP1A is possible without the use of radical techniques; but there are barriers to crossing, chromosomes pair poorly or not at all, many or most hybrids are sterile and some may be weak and difficult to bring to maturity. Nevertheless there are at least some fertile F_1s.

GP3: tertiary gene pool, consisting of those biological species that can be crossed with the crop but from which gene transfer is possible only through the use of radical techniques such as embryo culture, grafting or tissue culture, doubling the chromosome number, or using bridging species. Two GP3 groups may be distinguished --

GP3A: species producing viable but sterile hybrids with GP1A;

GP3B: species producing inviable hybrids.

5. Regions of diversity of domesticated forms (GP1A) of each crop are identified with the codes
   D1: primary region of diversity;
   D2: secondary region(s) of diversity.

Unless stated otherwise this information is from Zeven & de Wet (1982).
Note: regions of diversity are not necessarily centres of origin.

ALLIACEAE

Allium cepa L. onion & shallot $350m (92/08)

GP1A: D1 South-central Asia (nw India, Pakistan, Afghanistan, s USSR, w China).

D2 Mediterranean region (s Europe, especially Greece; n Africa; w Asia, especially Turkey).

Many locally adapted cvs in Asia, Africa, Central and South America and Europe being replaced by modern cvs. Also a rapid turnover of modern (bred) open-pollinated cvs of Asia, Europe and USA, particularly with the introduction of F_1 hybrids (which have a very narrow genetic base). In several countries important old cvs have been lost; in Western Europe most cvs now derived from a single type; in Egypt winter onion crop consists of a single cv (Astley, Innes & van der Meer 1982). Genetic base of US hybrids quite narrow (McCollum 1976). In Sudan, however, the local onion landraces have "competed better than the introduced" cvs, showing marked superiority in keeping quality; as a result "they have maintained their complete domination in all onion producing areas", and the introduced cvs are no longer grown (Hassan *et al*. 1983).

GP1B: possibly *A. vavilovii* M.Pop. & Vved., but identity uncertain; wild: s USSR (Turkmen SSR), n Iran (McCollum 1974). No data on status.

GP2: *A. galanthum* Kar. & Kir.; *A. psekemense* B.Fedtsch.; both wild: Central Asia (McCollum 1971). *A. psekemense* is listed in the Red Data Book of USSR (USSR Ministry of Agriculture 1978).

GP3A: *A. altaicum* Pall.; wild: n Mongolia, adjacent USSR (McCollum 1976). *A. oschaninii* O.Fedtsch.; wild: n Iran, Afghanistan, s USSR, w China (Pamir Alai & Tien Shan) (McCollum 1974). Possibly *A. farctum* Wendelbo (wild: Afghanistan, Pakistan) but crossability not determined (McCollum 1976). No data on status of wild species. A. fistulosum L.; domesticated (Japanese bunching onion): D1 Japan (Astley, Innes & van der Meer 1982). No data on status. "Local cvs should be identified and collected as soon as possible throughout the crop distribution area [USSR, China, Japan, Southeast Asia], especially in China (the centre of origin) and in Japan (the centre of diversity)" (Astley, Innes & van der Meer 1982).

CHENOPODIACEAE

Beta vulgaris L.	beet, beetroot, chard, sugarbeet	$1,036m (100/0)

GP1A: D1 Mediterranean region.

D2 Central Asia.

While undoubtedly many open-pollinated multigerm populations have been lost, modern hybrids still contain much genetic variation and "there is no substantial evidence that the present gene pool ... is particularly narrow or in immediate danger of being exhausted"; however, the hybrids derive from a single source of cytoplasmic male sterility (CMS) and thus have almost completely uniform cytoplasms; three new sources of apparently stable CMS have been identified in wild GP1B beets (in Morocco, Turkey and Yugoslavia) but have not yet been used in commercial hybrid production (Bosemark 1979). The disappearance of local cvs is accelerating in Greece (Cortessi, de Bock & Denton 1981).

GP1B: B. adanensis Pamuk.; wild: e Mediterranean. B. atriplicifolia Rouy; wild: Spain. Diploid B. macrocarpa Guss.; wild: Mediterranean region. B. maritima L.; wild: Europe, North Africa, West Asia. B. patula Ait.; wild: Madeira Is. (Buttler 1977; Coons 1954 & 1975). B. maritima is in danger of genetic erosion in some coastal parts of Greece due to the "rapid expansion of building associated with tourism" (Cortessi, de Bock & Denton 1981), in coastal Sicily due to rapid and intensive development associated with tourism, and in inland Sicily due to overgrazing by goats (Toll & Hendriksen 1982).

GP2: Tetraploid *B*. *macrocarpa*; wild: Canary Is. (Buttler 1977). No data on status.

GP3A: Species in section Corollinae (all wild): *B*. *foliosa* Hausskn. (Turkey); *B*. *lomatogona* Fisch. & Mey. (West Asia); *B*. *macrorhiza* Stev. (West Asia); *B*. *trigyna* Wald. & Kit. (incl. *B*. *corolliflora* Zos.) (Southeast Europe & West Asia) (Coons 1954 & 1975; Savitsky 1969). Species in section Procumbentes (Patellares) (all wild): *B*. *patellaris* Moq. (s Spain, Canary Is., Madeira Is., Morocco, Western Sahara); *B*. *procumbens* Chr. Sm. (Canary Is.); *B*. *webbiana* Moq. (Canary Is.) (Coons 1954 & 1975; Gaskill 1954; Oldemeyer & Brewbaker 1956; Savitsky 1975; Speckmann & de Bock 1982). There "appears to be little or no danger in the future of a great reduction" in the frequency and distribution of the two narrowly endemic species, *B*. *procumbens* and *B*. *webbiana* (Ford-Lloyd, Jackson & Santos Guerra 1982). (The remaining *Beta* species, *B*. *nana* Boiss. & Held., is confined to a few mountains in Greece [incl. Mount Olympus National Park] and is classified as rare by IUCN [Lucas & Synge 1978]; it has not been crossed with *B*. *vulgaris* and has no known agronomic potential.) No data on the status of the other species listed above.

COMPOSITAE

Helianthus *annuus* L. sunflower $581m (99/01)

GP1A: D1 USA.

D2 USSR.

Several American Indian varieties have already disappeared (Heiser 1976).

GP1B: *H. annuus*; wild: s Canada, USA, n Mexico (Heiser *et al.* 1969). Beard (1981) and Fick (1978) note the disappearance of wild *Helianthus* gene pools, chiefly due to habitat loss. Although wild *H. annuus* is widespread and abundant, agronomically useful characteristics are not distributed uniformly throughout the species and gene pools of particular value may be at risk.

GP2: Most other species in section Annui (all wild, although *H. argophyllus* & *H. debilis* are also domesticated ornamentals): *H. argophyllus* T. & G. (USA: TX); *H. bolanderi* Gray (USA: CA, OR); *H. debilis* Nutt. (e USA); *H. exilis* Gray (USA: CA); *H. neglectus* Heiser (USA: NM, TX); *H. niveus* (Benth.) Brandegee (sw USA, n Mexico); *H. paradoxus* Heiser (USA: NM, TX); *H. petiolaris* Nutt. (S Canada, USA, n Mexico); *H. praecox* Engelm. & Gray (USA: TX); two other species in this section -- *H. anomalus* Blake (USA: AZ, UT) and *H. deserticola* Heiser (USA: AZ, NV, UT) -- are very difficult to germinate (as are their hybrids with *H. annuus*) and perhaps should be assigned to GP3 (Beard & Chandler 1982; Heiser 1978; Rogers, Thompson & Seiler 1982; Whelan 1978a). Two of the above species, *H. anomalus* and *H. deserticola*, and two subspecies, *H. debilis debilis* (FL) and *H. praecox hirtus* (TX), are considered rare (Rogers, Thompson & Seiler 1982). Populations of *H. anomalus* in the Hopi Indian Reservation in nw AZ appear to be adapted to traditional Hopi agricultural practices and are protected or tolerated by Hopi farmers (Nabhan & Reichhardt nd). *H. exilis* is an endangered species, threatened by habitat disturbance and competition from more tolerant species; *H. nuttallii parishii* may already be extinct, its only known habitat having been destroyed by the expansion of Los Angeles; *H. paradoxus* is endangered -- two of its four known populations have been destroyed by highway construction and improvement (Jain, Olivieri & Fernandez-Martinez 1977; Rogers, Thompson & Seiler 1982). The remark on the status of wild *H. annuus* (GP1B) also applies to *H. petiolaris*.

GP3A: So far 10 species (7 wild; 3 wild and domesticated -- the ornamentals, *H*. *decapetalus* and *H*. *maximiliani*, and the Jerusalem artichoke, *H*. *tuberosus*): *H*. *gracilentus* Gray (USA: CA; n Mexico); *H*. *angustifolius* L. (e USA); *H*. *decapetalus* L. (se Canada, e USA); *H*. *hirsutus* Raf. (e USA, n Mexico); *H*. *pauciflorus* Nutt. [= *H*. *rigidus* Desf.] (s Canada, USA); *H*. *giganteus* L. (s Canada, ne USA); *H*. *grosserratus* Mart. (e USA); *H*. *maximiliani* Schrad. (s Canada, USA). Four of these species (*H*. *hirsutus*, *H*. *pauciflorus*, *H*. *strumosus*, *H*. *tuberosus*) produce almost sterile hybrids with *H*. *annuus* and could be included in GP2 (Heiser *et* *al*. 1969; Rogers, Thompson & Seiler 1982; Whelan 1978a & 1978b). No data on status.

Lactuca *sativa* L.	lettuce	$566m (100/0)

GP1A: D1 Mediterranean region (Ryder & Whitaker 1976).
No data on status. In the US there has been a rapid turnover of cvs: for example, of the 107 distinct cvs grown in 1904 only three are readily available today (Ryder 1980). In Syria 80% of cos (romaine) is local material (George 1984).

GP1B: *L*. *serriola* L.; wild: Europe, USSR, w Asia, n Africa (Ferakova 1977; Lindqvist 1960; Whitaker 1969). No data on status.

GP2: *L*. *saligna* L.; wild: Europe, USSR, w Asia, n Africa (Ferakova 1977; Lindqvist 1960; Whitaker 1969). No data on status.

GP3A: *L*. *virosa* L.; domesticated (Europe) & wild: Mediterranean region (Ferakova 1977; Globerson, Netzer & Sacks 1980; Lindqvist 1960; Whitaker 1969). No data on status.

CRUCIFERAE

Brassica *oleracea* L.	cole crops (broccoli, Brussels sprouts, cabbage, cauliflower, Chinese kale, collards, kale, kohlrabi)	$433m (96/04)

GP1A: D1 Mediterranean region & Northern Europe (IBPGR 1981b).

Broccoli: Italian gene centre threatened by expansion of the export cauliflower industry; genetic base of "Calabrese" (green-sprouting annual broccoli) is narrow and needs enlarging; biennial types of Northwest Europe under no immediate threat (IBPGR 1981b).

Brussels sprouts: advent of F_1 hybrids caused "massive genetic erosion"; however, the crop has few unique genes, due to its recent origin (18th Century), and most types have been relatively well collected (IBPGR 1981b).

Cabbage: increasing use of F_1 hybrid cvs threatens genetic resources in many parts of the world (IBPGR 1981b). There is "wide diversity" of cabbage in Syria (George 1984).

Cauliflower: the Italian gene centre "has not been assessed or collected" and is rapidly eroding as the export cauliflower industry develops there; in several parts of Northern Europe Australian cvs have virtually replaced traditional autumn-maturing stocks; there is still a wide range of commercial and growers-own cvs of British and Dutch biennial types (IBPGR 1981b). In Syria less than 5% of cauliflower is local (George 1984).

Chinese kale: no data on status (the crop is quite uniform, probably because of the absence of gene flow from other cole crops) (IBPGR 1981b).

Kale (incl. collards): there is relatively little danger of breeding or other agricultural developments causing major erosion of northern European types (IBPGR 1981b).

Kohlrabi: the crop is rather diverse but there are no data on status (IBPGR 1981b).

GP1B: Wild species of the n=9 cytodeme: B. bourgaeui (Webb) O.Kuntze (Canary Is.); B. cretica Lam. (Greece); B. hilarionis Holmb. (Cyprus); B. insularis Moris. (Corsica, Sardinia, Tunisia); B.

macrocarpa Guss. (Italy: Egadi Is.); *B. oleracea* L. (England, France, Spain); *B. montana* Pourr. (= *B. robertiana* Gay) (Spain, France, Italy); *B. rupestris-villosa-incana* complex (Italy, Yugoslavia) (Snogerup 1979 & 1980; Stork, Snogerup & Wuest 1980). IUCN (1980a & 1980b) classifies two of the above taxa as endangered -- *B. bourgaeui* (considered extinct until the recent rediscovery of two individuals [IBPGR 1981b] and *B. macrocarpa* (a highly restricted endemic); two as vulnerable (= threatened in the US) -- *B. hilarionis* and *B. insularis*; and one as rare -- *B. villosa* Biv. (Italy). IBPGR (1981b) states that many populations of the other species (*B. cretica*, *B. oleracea*, *B. montana*) are endangered, although the species themselves are not. However, Gustafsson, Gomez-Campo & Zamanis (1983) surveyed *B. cretica* and report that most populations of this species are large and inaccessible enough to be in no immediate danger of extinction, "even from human collectors".

GP2: None?

GP3: Possibly about 100 species in *Brassica* and allied genera (Harberd 1976), of which only the main domesticated species (and their wild GP1B forms) are reviewed here.

B. carinata Braun, Ethiopian mustard. GP1A:D1 Ethiopia. GP1B: none known. (Prakash & Hinata 1980). "Much of the genetic variation would appear to be conserved" (IBPGR 1981b).

B. juncea (L.) Czern., brown mustard. GP1A: D1 Indian subcontinent (oilseed types), China (leaf and root vegetables) (IBPGR 1981b; Li 1980). GP1B: West Asia (Prakash & Hinata 1980). No data on status.

B. napus L., fodder rape, oilseed rape, rutabaga. GP1A: D1 Northern Europe. GP1B: none known (IBPGR 1981b; McNaughton 1976a). Fodder rape "appears to be in serious danger of extinction"; most of the genetic variation of oilseed rape and rutabaga appears to be conserved (IBPGR

1981b). Many of the older cvs of rutabaga have gone out of cultivation in recent years, due partly to lack of demand as better cvs become available and partly to the failure to meet legal requirements of "distinctiveness, uniformity and stability" associated with the National List System of the European Economic Community; as a result many of these cvs are in danger of extinction (Munro 1978).

B. nigra (L.) Koch, black mustard. GP1A: D1 Europe. GP1B: Europe, North Africa, West Asia (Prakash & Hinata 1980). Many landraces have been lost (although their genes may persist in wild populations) as the result of its virtual extinction as a crop in the 1950s following its replacement by B. juncea (IBPGR 1981b).

B. rapa L. (B. campestris L.), choi crops (Chinese cabbages [pai ts'ai, pak choi, choi sam, etc.], turnip, turnip oilseed rapes). GP1A: D1 China, India (Li 1981; Narain 1974; Prakash & Hinata 1980). GP1B: Europe and much of Asia (Li 1981; Prakash & Hinata 1980). Chinese cabbages: much of the open-pollinated material of Chinese cabbage sensu stricto (pai ts'ai, B. rapa ssp. pekinensis) in Japan and Korea has been lost following the introduction of F_1 hybrids; in China (the D1) cultivation of F_1 hybrids is expanding rapidly and "it may not be...long" before landraces and other open-pollinated cvs are irretrievably lost (IBPGR 1981b); no data on the status of other Chinese cabbages. Turnip: no data on status. Turnip oilseed rapes: there is limited diversity in the European types -- biennial winter turnip rape and annual summer turnip rape; the former type is little grown now, and while the latter is important in Canada and northern Europe few landraces exist (although these are highly variable) and the genetic base of modern cvs is narrow; by contrast, there is considerable variation in the Asian turnip rapes (sarson, toria, yau choi) but apparently no data on their status (IBPGR 1981b).

Raphanus sativus L., radish. GP1A: D1 Europe and South and East Asia (IBPGR 1981b). GP1B: R. landra Moretti (Southern Europe); R. maritimus Smith (Mediterranean region and Atlantic Europe); R. raphanistrum L. (Europe and West Asia) (Banga 1976; Harberd 1972; Lewis-Jones, Thorpe & Wallis 1982; Zeven & de Wet 1982). No data on status.

Sinapis alba L. (= Brassica hirta Moench), white mustard, yellow mustard. GP1A: D1 Europe. GP1B: Mediterranean region (Hemingway 1976). No data on status.

B. juncea (Downey, Klassen & Stringham 1980), B. napus (McNaughton 1976a).

B. rapa (Ellerstrom 1978; McNaughton 1976b; Nishi 1981), R. sativus (Ellerstrom 1978; McNaughton 1976c) and S. alba (Downey, Klassen & Stringam 1980) are in the GP3A of B. oleracea.

General comment on genetic erosion in B. oleracea and B. rapa: "The desire of vegetable growers throughout the world for F_1-hybrids is resulting in the replacement of most landraces and open-pollinated cultivars ... [of these two species]. This replacement is virtually complete in Japan, Korea, North and South America and northern Europe. Very significant losses have occurred during the past 20 years in China, perhaps the last great resource of genetic diversity in oriental brassica vegetables; F_1-hybrids are now coming into wide use" (IBPGR 1981b).

EUPHORBIACEAE

Hevea brasiliensis (A.Juss.) Muell.-Arg.	rubber	$803m (0/100)

GP1A: D1 Southeast Asia (but the diversity of the domesticated gene pool is very small compared with that of the GP1B (Subramaniam & Ghani 1975). There has been an unknown amount of genetic erosion, due mainly to narrow breeding objectives (intensive selection for high yield potential, with little attention paid to "secondary characters" such as vigour and disease resistance) (Majid & Hendranata 1975; Wycherley 1969). "Further loss of available germplasm is bound to occur if only a few elite clones are planted" (Subramaniam & Ghani 1975).

GP1B: H. benthamiana Muell.-Arg.; wild: Brazil, Colombia, Peru, Venezuela. H. brasiliensis; wild: Bolivia, Brazil, Colombia, Peru, Venezuela. H. camporum Ducke; wild: Brazil. H. guianensis Aubl.; wild: Bolivia, Brazil, Colombia, French Guiana, Guyana, Peru, Suriname, Venezuela. H. microphylla, Ule; wild: Brazil, Colombia, Venezuela. H. nitida Muell.-Arg.; wild: Brazil, Colombia, Peru. H. pauciflora Muell.-Arg.; wild: Brazil, Colombia, Guyana, Peru, Venezuela. H. rigidifolia Muell.-Arg.; wild: Brazil, Colombia, Venezuela. H. spruceana Muell.-Arg.; wild: Brazil. Some of these species may be in GP2 (Majid & Hendranata 1975; Ong & Rao 1981; Seibert 1947; Wycherley 1976). H. brasiliensis in basin of Madeira River (Brazil) "under stress from human population pressure" (IBPGR 1984).

GRAMINEAE

Avena sativa L.	oats	$306m (99/01)

GP1A: D1 Europe (Holden 1976).
Improved cvs are replacing local material in Turkey "but not at a rapid rate" (Kjellqvist 1973).

GP1B: Hexaploid species: A. atherantha Presl; wild: Mediterranean region. A. fatua L.; a widespread and noxious weed. A. hybrida Peterm.; wild: West and Central Asia (also a European weed). A. occidentalis Dur.; wild: Azores, Canary Is., Madeira Is., North Africa, Saudi Arabia. A. sterilis L.; wild: Mediterranean region and West Asia (also a widespread weed). A. trichophylla C.Koch; wild: Mediterranean region and West Asia. (Baum 1977; Zohary 1971). All of these species are weedy and readily persist in and beyond their native areas (Zohary 1971); genetic erosion seems unlikely. However, Baum *et* *al*. (1972) remark that such species are susceptible to overgrazing, which they escape by colonizing cultivated fields where grazing is prevented. Genetic erosion could result either from overgrazing and other destruction of primary habitats (such as oak parkland) or from clean cultivation of agricultural habitats.

GP2: Two wild tetraploid species: *A*. *maroccana* Gdgr. (Morocco); *A*. *murphyi* Ladiz. (Spain). Four domesticated diploid species: *A*. *brevis* Roth (Western Europe); *A*. *hispanica* Ard. (Spain & Portugal); *A*. *nuda* L. (Europe); *A*. *strigosa* Schreb. (Europe). Seven wild diploid species: *A*. *canariensis* Baum, Rajhathy & Sampson (Canary Is.); *A*. *damascena* Rajhathy & Baum (Syria); *A*. *hirtula* Lag. (Algeria, Morocco, Spain); *A*. *longiglumis* Dur. (Mediterranean region); *A*. *lusitanica* (Tab. Mor.) Baum (Western Europe, North Africa); *A*. *matritensis* Baum (Western Europe, Northwest Africa); *A*. *wiestii* Steudel (Mediterranean region, West & South-Central Asia; also a weedy tetraploid). (Baum 1977; Holden 1976; Ladizinsky & Zohary 1971). *A*. *murphyi* and *A*. *damascena* are rare endemics (*A*. *maroccana* and *A*. *canariensis*, also endemics, are locally common) (Baum 1977). Cultivation of the domesticated species has either been abandoned or is limited and local, the species persisting as weeds (Baum 1977).

GP3: Four tetraploid species: *A*. *abyssinica* Hochst.; domesticated: Ethiopia. *A*. *barbata* Pott; a widespread weed (chiefly Europe, North Africa, West Asia). *A*. *macrostachya* Bal.; wild: Algeria. *A*. *vaviloviana* (Malz.) Mordv.; an Ethiopian weed. Three diploid species: *A*. *clauda* Dur.; a weed of the Mediterranean region and West Asia. *A*. *eriantha* Dur.; wild: Northwest Africa, Greece, West Asia. *A*. *ventricosa* Bal.; wild: North Africa, West Asia. (Baum 1977; Holden 1976; Ladizinsky & Zohary 1971). *A*. *macrostachya* is a rare endemic (Baum 1977). Overgrazing is seriously affecting important areas of genetic diversity in *A*. *clauda*, *A*.

eriantha and A. ventricosa; however, where grazing pressure is diminishing due to abandonment of livestock raising for other economic pursuits, the former two species at least are showing signs of recovery (Baum 1977; Baum et al. 1972).

Hordeum vulgare L. barley $780m (94/06)

GP1A: D1 Ethiopia, West Asia (especially Turkey & Iran), South-central and East Asia (Nepal, China, Korea, Japan) (Harlan 1979; Witcombe 1975).

D2 North Africa, Europe.

In Ethiopia there is a danger that important and useful populations will be discarded in the future and replaced by bred cvs (Harlan 1976). In Turkey improved cvs (mainly of Turkish origin) are rapidly replacing local material; an intensive barley improvement programme is being carried out (early 1970s), and the rate of replacement is expected to rise sharply (Kjellqvist 1973). In Iran, although improved cvs are becoming available, unimproved native varieties still prevail, the same applies to Iraq and Syria (where the landraces appear to be few but variable) (Frankel 1973). In Europe, North America and other regions of "advanced agriculture" landraces have been "virtually wholly displaced" by pure-line cvs (Harlan 1976). In Nepal no more than 10% of the barley area has been sown to improved cvs; the rest is under traditional types (IBPGR 1978c). In Libya genetic erosion is evident in the Cirenaica area, and only a few local barleys can be found in very remote areas (Lehmann & Hammer 1983). In Ethiopia there is no apparent threat of genetic erosion of local germplasm from the introduction of improved material (Toll 1980) (compare statement by Harlan at beginning of this paragraph).

GP1B: H. spontaneum C. Koch; wild (weedy forms also occur): Greece, Cyprus, Libya, Egypt, West Asia to Pakistan (Harlan 1979; Harlan & Zohary 1966). No data on status.

GP2: None known.

GP3: Potentially large, including several other (but not necessarily all) *Hordeum* species and species in other genera such as *Leymus* and *Secale* (National Council on Gene Resources 1982; Price 1979). North American GP3 species (all wild) with characteristics of potential value in barley improvement include *H. brachyantherum* Nevski (w Canada & USA); *H. jubatum* L. (Canada USA, Mexico): *H. pusillum* Nutt. (w Canada, USA, Mexico); *Leymus mollis* (Trin.) Pilger (Canada, n USA, East Asia) (Schooler 1962, 1967 & 1980; Schooler & Anderson 1979 & 1980). No data on status.

Oryza sativa L.	rice	$1,519m (100/0)

GP1A: Indica, sinica (japonica), javanica and intermediate types--

D1 India (especially ne), Bangladesh, Burma, Thailand, Laos, Vietnam, China (especially s) (Chang 1976a).

D2 Nepal, Sri Lanka, Kampuchea, Indonesia, Philippines (Chang 1976a; Chang, Adair & Johnston 1982).

The following notes on status and trends are from IRRI (1978), updated by Chang, Adair & Johnston (1982) and others as indicated (see also discussion, pages 1.9-1.15) --

Bangladesh: most cvs are indicas, of which there are four agro-ecological groups -- *aus*, transplanted *aman*, broadcast *aman*, and *boro*; there is little genetic erosion in the *aus* and broadcast *aman* groups, but traditional cvs of the other two groups are being replaced by modern cvs.

Burma: there are about 2,000 cvs; the rate of replacement by modern cvs is slow.

China: traditional cvs are indicas and sinicas (japonicas); replacement of traditional cvs has been rapid since 1959-63 when the semidwarfs were developed and 1978 with the extensive adoption of F_1 hybrids; in Taiwan traditional indica cvs "practically vanished after Taichung Native 1 was released in 1961" (Chang, Adair & Johnston 1982).

India: genetic erosion has been rapid in the irrigated areas (Chang, Adair & Johnston 1982). Until recently 5,500 cvs were grown, of which 700 were improved cvs (mostly selections from local stocks); since the early 1960s landraces and local selections have been replaced by the IRRI cvs and equivalent cvs bred in India in substantial areas, especially in the plains; replacement is slow but accelerating in the tribal hill areas (Singh 1973).

Indonesia: traditional cvs are indicas and javanicas; rate of replacement by modern cvs has been rapid; genetic erosion "has reached a critical point in Indonesia's rice production centers due to the spread of improved varieties" (IRRI 1978); from 1971-72 to 1979-80 the proportion of the rice-growing area grown to traditional cvs fell from about 45% to 30%, while the area under modern cvs increased from 31% to about 68% (the remaining percentages being occupied by local improved cvs developed prior to 1968); the rate and extent of replacement varies greatly with region (Bernsten, Siwi & Beachell 1981) -- see also discussion.

Iran: all cvs are indicas; little genetic erosion has occurred, but the introduction of improved cvs and irrigation and drainage projects "continually threaten" to replace traditional cvs.

Japan: all cvs are sinicas (japonicas); 20 leading cvs are grown on about 60% of the rice area; in 1962 1,302 local cvs were collected from farmers' fields. Culture of many of these cvs had stopped 2-3 years prior to their collection; many of those still in cultivation have special culinary qualities (Nagamatsu & Omura 1975) -- see also discussion.

Kampuchea: adoption of modern cvs negligible (Chang, Adair & Johnston 1982); but many local cvs lost as a result of war (Swaminathan 1984).

Korea (Republic of): all native cvs are sinicas; modern indica-sinica intermediates have spread rapidly since 1971 and now occupy 60-75% of the rice area (Chang, Adair & Johnston 1982).

Laos: the spread of modern cvs was negligible in the early 1970s (apparently no more recent data) (Chang, Adair & Johnston 1982).

Malaysia: modern cvs grown on 53% of rice area by 1977/78 (Herdt 1981).

Nepal: genetic erosion is slow overall, with local cvs still being grown on more than 80% of the rice area, predominating in the hills; however, in the Kathmandu and Chitawan valleys modern introduced cvs occupy 75% (more than 80%, according to Chang, Adair & Johnston 1982) of the rice area and genetic erosion is serious.

Pakistan: most cvs are indicas; modern cvs bring a great danger of genetic erosion, occupying 71% of the rice area in Sind, 40% in Baluchistan, 19% in North-West Frontier, and 13% in Punjab; Baluchistan, Azad Kashmir, and several districts of North-West Frontier are still rich in genetic diversity.

Philippines: most cvs are indicas, some are javanicas; genetic erosion apparently advanced--of the tropical Asian countries, Philippines has the largest proportion (about 90%) of the rice area grown to modern high-yield cvs (but a substantial proportion of replaced cvs were foreign ones introduced before and after World War II) (Chang, Adair & Johnston 1982).

Sikkim: modern cvs have been introduced but there is still considerable local variability (Arora 1981).

Sri Lanka: genetic erosion has been rapid since the early 1970s; locally improved cvs are grown on more than 90% of the rice area (Chang, Adair & Johnston 1982).

Thailand: genetic erosion in upland (wet season) rice is rather slow; the genetic diversity of lowland and floating rice is in serious danager of reduction as modern cvs are replacing local cvs; this process is compounded by the extension and improvement of the irrigated area.

Vietnam: "genetic erosion had already taken its toll in the late 1960s and early 1970s when the semidwarfs [modern improved cvs] spread rapidly in the favored areas" (Chang, Adair & Johnston 1982).

Central and South America: genetic erosion almost 100% in irrigated and mechanized upland areas; there has been almost no erosion in the unmechanized upland areas.

USA: many of the cvs presently grown have rather similar genetic backgrounds.

GP1B: *O. sativa* weed form *spontanea* (Indian subcontinent, Southeast Asia, s China); wild annual *O. nivara* Sharma & Shastry (Indian subcontinent, Southeast Asia, s China, n Australia); wild perennial *O. rufipogon* Griff. (Indian subcontinent, Southeast Asia, s China, New Guinea, tropical America) (Chang 1975, 1976a & 1976b). Construction of irrigation and drainage facilities, highways, industries and housing, together with the intensification of agriculture (which discourages the persistence of weedy relatives) and the introduction of improved cvs (which facilitates roguing), have destroyed populations of all three taxa and threaten others (Chang 1975 & 1976b; IBPGR 1982a). Wild rices became extinct in Taiwan in the 1970s (Chang, Adair & Johnston 1982). Despite loss of habitat through deforestation (Whyte 1975), wild rices (chiefly form *spontanea*) are still abundant in s China: a survey of one county (Boluo) found that populations at 13 or 22 localities had remained unchanged (since when was not specified), 3 had been reduced slightly, and 6 had been reduced to a half or more of the original size (Chen, Liang & Yu 1980).

GP2: Domesticated *O. glaberrima* Steud. (West Africa; D1 upper Niger River & Niger inland delta) and its GP1B: *O. glaberrima* weed form *stapfii* (West Africa); wild annual *O. barthii* A.Chev. (West Africa, e to Sudan & s to Tanzania & Zambia); wild perennial *O. longistaminata* Chev. & Roehr. (tropical Africa incl. Madagascar) (Bezancon *et al*. 1978; Chang 1976b). *O. sativa* cvs are quickly replacing *O. glaberrima*; the area occupied by African rice is decreasing and genetic diversity in the species is being eroded (IRRI 1978; Sharma & Steele 1978). A large number of landraces of *O. glaberrima* can still be found in Guinea (Bezancon, de Kochko & Goli 1984). *O. longistaminata* populations have been reduced in number (IRRI 1978).

GP3: Includes at least six other *Oryza* species (all wild); genome designations are given in parentheses (GP1 & GP2 spp. are AA): *O. australiensis* Domin. (EE) (Australia), *O. brachyantha* Chev. & Roehr. (FF) (West & Central Africa); *O. latifolia* Desv. (CCDD) (Central & South America); *O. minuta* (BBCC) (Southeast Asia); *O. officinalis* Wall. (CC) (South & Southeast Asia, s China); *O. punctata* Kotschy (BB, BBCC) (Africa) (Oka pers. comm. 1981). Assumed to include remaining *Oryza* species also (all wild): *O. alta* Swallen (CCDD) (Central & South America); *O. eichingeri* Peter (CC, BBCC) (East & Central Africa); *O. grandiglumis* (Doell.) Prod. (CCDD) (South America); *O. granulata* Nees & Arn. (?) (South & Southeast Asia); *O. longiglumis* Jansen (?) (New Guinea); *O. meyeriana* (Zoll. & Mor.) Baill. (?) (Southeast Asia, s China); *O. ridleyi*

Hook.f. (?) (Southeast Asia); O. schlechteri Pilger (?) (New Guinea) (Chang, Marciano & Loresto 1977; Khush 1975; Khush pers. comm. 1982). No data on status, except that in the Philippines O. meyeriana and O. minuta are said to be abundant (IRRI 1978).

Saccharum officinarum L. sugarcane $2,550m (22/78)

Modern commercial cvs of sugarcane are complex hybrids involving the original GP1A species S. officinarum (2n=80), wild GP2 species S. spontaneum L. (2n=40-128), the horticultural hybrid group (GP2) "barberi-sinense" (2n=82-124), and to a limited extent the wild GP1B species S. robustum Brandes & Jeswiet (2n=60,80). The hybrid cvs (2n=100-125) are often designated as "Saccharum spp. hybrid", although their S. officinarum content is very high (in female S. officinarum x male S. spontaneum crosses, the somatic chromosome number of S. officinarum is transmitted intact and then increased in the first backcross, while the chromosome complement of S. spontaneum is first halved and then further reduced [to about a quarter]). It seems appropriate to retain S. officinarum as the name of the crop (GP1A) but expand the GP1B to include all other Saccharum taxa. The GP2 would include genera, such as Miscanthus and Imperata, that apparently can be hybridized more readily with the hybrid cvs than with the noble canes of S. officinarum L. (Price 1957, 1965a & 1965b; Roach 1971; Simmonds 1976b).

GP1A: D1 New Guinea (Simmonds 1976b).

D2 Oceania, Southeast Asia (Simmonds 1976b).

Increasing availability of crystalline sugar and other changes in life style associated with development have resulted in the abandonment of native gardens where traditional cvs are maintained (Berding & Koike 1980; Daniels, Smith & Paton 1975). Genetic erosion high in n India, low in s Philippines and Indonesia (Kalimatan, Irian Jaya) (IBPGR 1982b).

GP1B: Horticultural hybrid group "barberi-sinense": D1 ne India to s China (Simmonds 1976b). *S. robustum* (incl. *S. sanguineum* [Grassl] Grassl); wild: Indonesia (Kalimantan to Irian Jaya), Papua New Guinea, Solomon Is., New Hebrides (Price 1965a). *S. spontaneum*; wild (incl. weedy types): tropical Africa, North Africa and insular Mediterranean, West Asia, South-Central Asia, Indian subcontinent, East and Southeast Asia, New Guinea to Fiji (Panje & Babu 1960). Valuable germplasm of wild *Saccharum* and related species has already been lost as a result of urbanization and agricultural expansion in Malaysia, Indonesia, Papua New Guinea, and India (Daniels, Smith & Paton 1975; Sreenivasan, Palanichamy & Koppar 1982). Such pressures, combined with forestry and mining developments, continue to erode the wild gene pools of Southeast Asia and New Guinea (Berding & Koike 1980). Genetic erosion high in "barberi-sinense" in Burma and Thailand, *S. robustum* in New Guinea (both Irian Jaya and Papua New Guinea), and *S. spontaneum* in n Pakistan and Iran; genetic erosion medium in *S. robustum* in Indonesia (Sulawesi, Seram, Halmahera, Buru); genetic erosion low in *S. spontaneum* in East Africa, Egypt, Turkey, Iraq, s USSR, Afghanistan, Bangladesh, India-China border, Burma, Thailand, Kampuchea, Laos, Vietnam, Malaysia (IBPGR 1982b).

GP2: Includes species in several other genera--*Eccoilopus*, *Erianthus*, *Imperata*, *Miscanthidium*, *Miscanthus*, *Narenga*, *Sclerostachya*, *Sorghum*, *Zea* (some may be GP3)--of which *Miscanthus* is currently considered the most useful in breeding (James 1980; Price 1957). Genetic erosion in *Miscanthus* is high in the Himalaya (Bhutan and Nepal), China, Southeast Asia and New Guinea (Irian Jaya) (IBPGR 1982b).

Sorghum bicolor (L.) Moench   sorghum & broomcorn $1,442m (99/01)

GP1A: Ssp *bicolor* (races bicolor, guinea, caudatum, kafir, durra and intermediates).
D1 Africa.

D2 India.

The extent of genetic erosion in Africa "varies enormously from region to region depending to a considerable degree on the amount of plant selection work and the success of extension programmes. The American types of hybrid grain sorghums are adapted only in South Africa and have not displaced indigenous landraces elsewhere. The impact in South Africa, however, may be considerable. In Upper Volta there has been a wide scale replacement of landraces by varieties selected out of them. The genetic erosion is probably considerable. Similar trends are underway in Sudan, Nigeria, Niger and Senegal, but as of the present moment the indigenous landraces are still to be found almost everywhere in Africa and the threat of serious genetic erosion is not immediate. There is evidence, however, that the situation could change drastically in the next few years" (Harlan 1973). Zimbabwe is still a valuable source of diversity, but indigenous material is being lost (due largely to replacement of sorghum by maize), and collection is of the highest priority (Toll & Gwarazimba 1983). In PDR Yemen there is no major danger of loss of germplasm as so far only the local cvs meet farmers' needs (Mu'Allem 1981).

GP1B: Ssp arundinaceum (spontaneous races arundinaceum, aethiopicum, virgatum, verticilliflorum, propinquum, shattercane): Africa (Harlan & de Wet 1972; de Wet, Harlan & Price 1976). Race arundinaceum is spreading as tropical forests are cut back (Harlan 1981); stands of race aethiopicum have disappeared from some sites in Sudan due to overgrazing associated with resettlement programmes; and stands of race verticilliflorum are being destroyed as additional areas of savanna are brought into cultivation (Harlan 1973 & 1975).

GP2: None known.

GP3: S. almum Parodi; domesticated (forage) and wild: Argentina. S. halapense (L.) Pers.; wild (Mediterranean region) and a noxious cosmopolitan weed (Cox pers. comm. 1981; Doggett 1964 & 1976). No data on status, but genetic erosion unlikely.

Triticum aestivum (L.) Thell.	common wheat, club wheat	$8,867m (100/0)
Triticum turgidum (L.) Thell.	macaroni (durum) wheat	

Gene pools are defined in terms of T. aestivum.

GP1A: All ssps of T. aestivum (hexaploids, genome AABBDD) (compactum, macha, spelta, sphaerococcum, vavilovii, vulgare).

D1 Southwest Asia.

D2 South-Central Asia, East Asia, Mediterranean region (North Africa).

Afghanistan: "When this survey was made [early 1970s], Afghanistan's wheats seemed safer from genetic erosion than almost any others. Since then, two years of drought, harvest failures, and catastrophic famine have drastically changed the picture. Thousands of tons of seed have been imported. Introduced varieties now predominate in many parts of the country. In mountain areas where previously only indigenous varieties have ever grown, introduced varieties have now widely replaced them. Afghanistan is a warning that genetic erosion does not follow a predictable course, and that genetic conservation programmes must never be relaxed, even in regions considered safe from genetic erosion" (Bennett 1973a).

Algeria: the native wheats are rapidly disappearing as they are replaced by introduced cvs (Bennett 1973b).

Bhutan: Indian cvs replacing native cvs (Chapman 1984).

Cyprus: native races persist, largely due to their incorporation in modern but rather variable cvs (Bennett 1973b).

Egypt: genetic erosion extremely high in the north; in the south landraces still provide a remarkable source of genetic variation that has not yet been eroded (Porceddu 1979). Most of the crop is composed of modern cvs (IBPGR 1981e).

Greece: genetic erosion is far advanced, even in the mountain villages; the percentage of primitive cvs in the Greek wheat crop fell from 80% in 1930 to 10% in the late 1960s; although there has been some agroecotypic differentiation in the introduced cvs, only rarely can genuine local races be found; these are now restricted to the most isolated mountain areas (Bennett 1973b). Genetic erosion severe with the introduction of new cvs, new techniques and new crops; much of the northwest coastland has recently changed from cereals to fruit and vegetable production (IBPGR 1981e).

India: during the 1940s and 1950s the mixed landrace populations of the northern plains (the main wheat area) were replaced by improved cvs derived from them; by the late 1960s these in turn had been replaced by the Mexican wheats, which have also spread to other wheat areas; very little variability remains in the plains, but local wheats still survive in pockets in the hill areas (Singh 1973).

Iran: in the Caspian Sea zone local populations and cvs have been completely replaced by introduced cvs, which also occupy 90% of the wheat area in Khuzistan (sw Iran); introduction programmes are increasing and threaten the diversity of native gene pools, although traditional cvs were still being grown under rainfed conditions in Azerbaidjan and Khurasan (nw & ne Iran respectively); "in many areas, crops sown to indigenous varieties are not only rare and indeed sporadic, but are not consistently grown in any areas, small as they may be" (Frankel 1973). Spelt (ssp spelta) cultivation, formerly practised in the high plateau of central Iran, disappeared completely between 1957 and 1972 (Kuchuck 1973).

Israel: traditional landraces being replaced by modern cvs (Chapman 1984).

Nepal: 80% of the wheat area is sown to improved cvs (IBPGR 1978c). Two distinct types of modern wheat--pre-Mexican and Mexican; the former, introduced 1950-1965, caused little genetic erosion of local germplasm (in fact it added to the diversity) because it was always grown side by side with the local cvs; in contrast, many farmers abandon local cvs within 2 years of starting to grow the Mexican (CIMMYT derived) cvs; local germplasm is disappearing rapidly, and is expected to go on doing so with the introduction of Mexican cvs with outstanding adaptability such as RR21 (Erksine & Bourgois 1979).

Netherlands: spelt (ssp spelta) is no longer cultivated, and the last remaining collection of Dutch landrace cvs was discarded and is now lost (Zeven 1979).

Pakistan: replacement of native populations by modern uniform cvs is very advanced and there appears to be "relatively little left to be salvaged" (Frankel 1973). Genetic erosion occurring in Baluchistan only in areas where irrigation is available; genetic erosion generally severe in Azad Kashmir due to introduction of Mexican lines (Chapman 1984).

Poland: local races are still grown and are also maintained as components of both recent and older cvs; the local races are highly variable (Hanelt 1979).

Spain: ssp *spelta* found mainly in the Asturias; old local cvs of ssp *vulgare* mainly confined to Galicia and Costa Vasca; seed of improved cvs widely distributed and genetic erosion is very much advanced (Porceddu 1979). Most of the crop in n Spain is composed of modern cvs but in some areas farmers continue to grow their traditional cvs (IBPGR 1981e).

Turkey: improved cvs have very nearly replaced local material; there are sporadic remnants in the Pontic and Taurus mountains (Kjellqvist 1973).

USSR: genetic erosion is high in Georgian SSR (Anonymous 1983; Chapman 1984).

PDR Yemen: the danger of losing germplasm is increasing due to the accelerated adoption of high response cvs during the last 7 years; with the introduction of mechanization, among other measures, "HYVs" will cover nearly 67% of the wheat area by 1984-85 (Mu'Allem 1982).

GP1B: None.

GP2: *T*. *zhukovskyi* Men. & Er. (hexaploid, genome AAAABB); domesticated: USSR (Georgia). *T*. *turgidum* (tetraploids, genome AABB); domesticated ssps *carthlicum*, *dicoccon*, *paleocolchicum*, *turgidum* (this last includes the durum cvs) D1 Mediterranean region, Southweat Asia; D2 Ethiopia, India; wild ssp *dicoccoides* (Korn.) Thell. (Southwest Asia). *T*. *timopheevi* Zhuk. (tetraploids, genome AAGG); domesticated ssp *timopheevi* Zhuk. D1 USSR (Georgia); wild ssp *araraticum* (Jakubz.) MK. (Turkey, Iraq, Iran, sw USSR). (Croston & Williams 1981; Feldman & Sears 1981; Heyne & Smith 1967; Morris & Sears 1967).

Data on status of *T*. *turgidum* domesticated ssps--

North Africa (general): plant breeding began in the 1900s using indigenous germplasm with the aim of saving gene blocks that (for example) conferred disease resistance and adaptations to local conditions; these new selections spread quickly, displacing old local populations over vast areas, including areas for which they were not selected; as a result, crop losses occurred due to septoriosis, a disease well tolerated by the landraces; subsequently the spread of cvs based on introduced germplasm substantially increased the danger of genetic erosion (Porceddu 1979).

Algeria: in the Atlas Mountains large areas administered by cooperatives are planted with one or few cvs; independent farmers grow the same cvs to be sure of selling their crop; except in a small zone where traditional mixtures of lines are still grown, only 3 indigenous cvs (modern varietally pure selections from landraces) are now cultivated. In the oases cultivation of wheat has declined sharply since 1965, due to increased cultivation of vegetables as a cash crop, emigration to towns, and abandonment of some oases because of the current drought cycle; in one region only 3 populations of wheat were found where 18 landraces were said to exist in the past. By contrast in the poor and isolated Ahaggar mountains in the south, there is little danger of genetic erosion: "the Touaregs prefer to cultivate the landraces instead of the new ones provided by the Ministry of Agriculture, because the former have a shorter cycle and other not well defined advantages ...[; the] Touaregs eat the seeds given from the State and continue to cultivate their own cultivars" (Porceddu 1979).

Egypt: genetic erosion in durum wheat is not yet severe due to the fact that few if any new cvs have been introduced (Porceddu 1979).

Ethiopia: severe genetic erosion observed in Arsi (only 2 local types remain in cultivation); genetic erosion not severe in Wollo, Shoa or Gemu Gofa (IBPGR 1981e).

Greece: as for T. aestivum; severe genetic erosion has already occurred; even in mountain areas where landraces persist, a few landraces have replaced the former rich spectrum of variation; in Crete the main plains are almost completely planted to fruit and vegetable crops and wheat is no longer grown; landraces are still grown in the mountains of Crete, but introduced cvs are increasing rapidly "because considerable amounts of seed have been and continue to be distributed by the agricultural banks and credit organization" (Porceddu 1979). Greece still a rich area of genetic variability for durum wheat (Chapman 1984).

India: the threat to local durum germplasm in central India is less than that to T. aestivum because the former grows under exacting unirrigated conditions, while the latter is grown mainly under irrigation for which many improved cvs are available (Arora & Koppar 1981).

Iran: there is a great deal of variation in populations in Kermanshah (the main durum growing province), but it is threatened by introduction programmes, which are increasing (Frankel 1973).

Italy: heavy genetic erosion but ssp dicoccon still cultivated (Chapman 1984). In Sicily many extensive areas grow only uniform cvs yet a surprising amount of genetic diversity still exists; in addition to local forms that can be found mixed with modern cvs, landraces still make up the bulk of the crop in the interior of the island, outside the major wheat area (Bennett 1973b).

Morocco: landraces seriously threatened (IBPGR 1984).

Libya: genetic erosion (in both T. turgidum and T. aestivum) is not great (Porceddu 1979). The Fezzan area is still rich (Lehmann & Hammer 1983).

Pakistan: in Azad Kashmir local durum races are almost extinct (Chapman 1984).

Spain: ssp dicoccon forms are now restricted to the mountains of the Asturias and Navarra; ssp turgidum, formerly widely grown throughout the country with numerous local forms, is now practically restricted to the mountains of Estremadura, Andalusia and Valencia (Porceddu 1979).

Tunisia: in the northern mountains and coastal ranges, and in most of the oases, field crops have been replaced by fruit and vegetables; old local cvs, similar to those of the Algerian Atlas, persist in El Kef province (the Tunisian Atlas) (Porceddu 1979).

Turkey: as for T. aestivum.

Yemen AR: introduced cvs will have an effect on the frequency of traditional cvs in cultivation; ssp dicoccon is rapidly declining in area as other crops with lower labour requirements replace it (IBPGR 1981e).

Data on status of other GP2 taxa--

T. turgidum dicoccoides: throughout much of its range in southwest Asia reduced by overgrazing to confinement to rocky or stony habitats where it can escape grazing pressure; where grazing is moderate it can be found growing in deep soil in large and dense stands, forming (with Avena sterilis and Hordeum spontaneum) the main annual grass component of the lush herbaceous cover of the submediterranean or semi-steppe herbaceous shrub formation; when grazing pressure is relaxed or ceases altogether, stands have recovered spectacularly (Zohary & Brick 1961).

T. timopheevi timopheevi: listed in the Red Data Book of USSR (USSR Ministry of Agriculture 1978).

T. timopheevi araraticum: listed in the Red Data Book of USSR (USSR Ministry of Agriculture 1978).

T. timopheevi and T. zhukovskyi have completely disappeared from cultivation over the last 2 decades (Anonymous 1983).

GP3: Large and expanding. Can be grouped into two, of which only the first will be listed here: (1) Triticum and Aegilops species; (2) species of other genera that have been successfully crossed with T. aestivum (Agropyron, Elymus, Haynaldia, Hordeum, Secale) (Feldman & Sears 1981; Sharma & Gill 1983; Zohary, Harlan & Vardi 1969). The first group can be further subdivided as follows:

i. Diploid donors of AA & DD genomes--T. monococcum L. (AA); domesticated ssp monococcum D1 Southwest Asia; wild ssp boeoticum (Boiss.) MK. (Southwest Asia, Bulgaria, Greece, Yugoslavia, Albania). T. urartu Tum. (AA); wild: Southwest Asia. Aegilops squarrosa L. (DD); wild: Southwest & South-Central Asia.

ii. Diploid species (all wild) with genomes homeologous to wheat: Aegilops bicornis (Forsk.) Jaub. & Sp. (Libya, Egypt, Israel); A. caudata L. (Southwest Asia, Greece); A. comosa Sibth. & Sm. (Greece, Turkey); A. longissima Schweinf. & Muschl. (Egypt, Israel, Jordan, Syria); A. mutica Boiss. (Turkey); A. searsii Feld. & Kis. (Israel, Jordan, Syria); A. speltoides Tausch (Southwest Asia); A. umbellulata Zhuk. (Southwest Asia, Greece); A. uniaristata Vis. (Yugoslavia, Albania, Greece, Turkey).

iii. Polyploid species (all wild) with one genome (DD) homologous to wheat: *A*. *crassa* Boiss. (Southwest & South-Central Asia); *A* *cylindrica* Host (South Europe to South-Central Asia); *A*. *juvenalis* (Thell.) Eig (Afghanistan, s USSR, Iran, Iraq); *A*. *vavilovii* (Zhuk.) Chenn. (Southwest Asia); *A*. *ventricosa* Tausch (South Europe, North Africa).

iv. Polyploid species (all wild) with genomes homeologous to wheat: *A*. *columnaris* Zhuk. (Southwest Asia); *A*. *kotschyi* Boiss. (Italy, Greece, North Africa, Southwest Asia); *A*. *lorentii* Hochst. (Algeria, South Europe, Southwest Asia); *A*. *ovata* L. (Mediterranean region, Iran); *A*. *triaristata* Willd. (Mediterranean region, Iran); *A*. *triuncialis* L. (Mediterranean region to South-Central Asia).

The acreage of *T*. *monococcum* *monococcum* (einkorn) in Turkey has decreased drastically "in recent years" (Kjellqvist 1973). In Italy there is heavy genetic erosion but *T*. *m*. *monococcum* is still grown in Basilicata, Campania and Molise (Chapman 1984). *T*. *m*. *boeoticum* is abundant on ungrazed sites at 400-1000m in ne Greece (IBPGR 1981e). *T*. *monococcum* *boeoticum* and *T*. *urartu* are listed in the Red Data Book of USSR (USSR Ministry of Agriculture 1978). *Aegilops* are abundant in Cyprus in open and disturbed habitats (Chapman 1984). In France there has been some loss of the habitats of *A*. *lorentii*, *A*. *ovata*, *A*. *triaristata* and *A*. *triuncialis* due to urbanization (more exploration is required) (Chapman 1984). In Greece there is a wealth of *Aegilops* spp in the Lassiti plain and the base of the Ida mountain (IBPGR 1981e).

Wild taxa (general): wild material is underrepresented in collections but is not seriously threatened by genetic erosion at present (IBPGR 1984); in Iran wild taxa "are the most threatened by overgrazing, deforestation and mechanization of agriculture" (IBPGR 1981e); northern Iraq is very rich in wild relatives of wheat--grazing intensity and agricultural pressures are very low and these species are "found over huge areas often in dense stands"; T. turgidum dicoccoides and T. urartu, for example, are locally widespread and abundant (FAO & IBPGR 1979).

Zea mays L. maize $14,296m (100/0)

GP1A: ssp mays

D1 Mexico, Central America (especially Guatemala), Andes, lowland Northern South America, lowland Southern South America, USA (Brown & Goodman 1977).

D2 West Indies, Amazon basin, China, Southeast Asia, Himalaya, Mediterranean region, Southwest Asia, Africa.

Much of the indigenous corn of the USA was replaced by modern hybrids prior to the implementation of an organized program of germplasm preservation (Brown & Goodman 1977).

In the West Indies many cvs have been replaced by modern hybrids: an estimated 90% of the maize grown in Jamaica consists of hybrid cvs (Brown & Goodman 1977). In Colombia 20% of the local germplasm is lost each year (IBPGR 1981f). Locally improved cvs are replacing traditional races in the oases of coastal desert Peru and Chile (Leon 1973a). In Bolivia "social and political disturbances" have led to the loss of some of the superior races, which are no longer found in the markets (Leon 1973b). Modern cvs are grown on only about 15% of the maize area in Nepal (IBPGR 1978c); and there appears to be no imminent danger of loss of traditional cvs in India (Singh 1973). In the Georgian SSR (USSR) local cvs can now be found in only a few localities (Anonymous 1984). In Kosovo and Macedonia (Yugoslavia) some farmers preserve local populations either because there are no suitable hybrids for cultivation at higher altitudes or because they prefer "domestic seed" for human consumption (Radovic & Zivcovic 1983). In Zimbabwe the genetic resources of traditional cvs have been lost due to replacement by locally bred hybrid cvs; "a few sweet corn and flint landraces were collected from farmers who showed a preference for their taste and storage ability" (Toll & Gwarazimba 1983).

GP1B: *Z*. *mays* ssp *mexicana* (Schrader) Iltis (Nobogame, Central Plateau and Chalco teosinte); essentially a weed of maize fields: Mexico. *Z*. *m*. ssp *parviglumis* Iltis & Doebley (Huehuetenango and Balsas teosinte); wild: Mexico, Guatemala. *Z*. *luxurians* (Durieu & Ascherson) Bird (Guatemala teosinte); wild: s Guatemala, Honduras. *Z*. *diploperennis* Iltis, Doebley & Guzman (diploperennial teosinte); wild: Mexico (Jalisco). (Doebley & Iltis 1980; Iltis *et* *al*. 1979; Wilkes 1967). The ranges of the two subspecies have been halved since 1900; they exist only as scattered populations, even the largest of which are under pressure from intensification of agriculture (cleaner cultivation), conversion to crops other than maize, and overgrazing by livestock; since 1960 the rate of population extinctions has been accelerating (Wilkes 1972 & 1977). The same pressures affect *Z*. *luxurians*, which of the three wild annual taxa is the most threatened with extinction in its native habitat, surviving only where it can escape grazing pressure along fencerows and among rock outcrops (Wilkes 1977).

GP2: *Z*. *perennis* (Hitchc.) Reeves & Mangelsdorf ([tetraploid] perennial maize); wild: Mexico (Jalisco) (Doebley & Iltis 1980). *Tripsacum* *dactyloides* (L.) L.; wild: USA, Mexico, Central America, South America. *T*. *floridanum* Porter; wild: USA (FL), Cuba. (de Wet 1979; de Wet, Gray & Harlan 1976; de Wet *et* *al*. 1981). Eleven other *Tripsacum* species may be in the GP2 or GP3 of maize but crossability data apparently are lacking. All are wild except for *T*. *andersonii* Gray (a domesticated forage species, which also occurs spontaneously in much of tropical America). Four of the wild species are narrow endemics: *T*.

bravum Gray (Mexico); *T. cundinamarce* de Wet & Timothy (Colombia); *T. maizar* Hern. & Randolph (Mexico); *T. zopilotense* Hern. & Randolph (Mexico). Distributions of the remainder are: *T. australe* Cutler & Anderson (South America); *T. fasciculatum* Trin. (Mexico, Guatemala); *T. lanceolatum* Rupr. (USA: AZ; Mexico); *T. latifolium* Hitchc. (Mexico to Costa Rica); *T. peruvianum* de Wet & Timothy (Ecuador, Peru); *T. pilosum* Scribn. & Merr. (Mexico to Honduras). Of the confirmed GP2 species, *Z. perennis* and *T. floridanum* are considered endangered (Ayensu & DeFilipps 1978; Lucas & Synge 1978). No data on the status of the other species.

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Diversity and Distribution of Wild Plants

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Diversity and Distribution of Wild Plants

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Abstract

Plant taxonomy, which studies the characterization, naming, and classification of plants, has been responsible for the development of our knowledge of the diversity and distribution of the estimated 265,000 species of plants that exist on Earth. About two-thirds of these plants occur in the tropics, where the survival of many is threatened by human activity, and the remainder in temperate zones. The inventory of plants is far from complete, particularly from tropical areas, where many species remain to be discovered and many others are known from one or a few specimens. The situation in temperate areas is better, though there is still no comprehensive treatment of the plants of North America. Information concerning the diversity of plants is scattered in hundreds of thousands of published books and journal articles, and electronic data banking of this information is just beginning. Advances in our knowledge of plant diversity are hampered by a lack of trained taxonomists (the world-wide pool should be at least eight times its current size), lack of adequate facilities to store and study specimens, and the lack of sufficient specimens to properly access diversity and distribution. Knowledge of this kind is essential to a broad spectrum of basic and applied research and is used by those who enjoy botany as a leisure time activity as well. Conservation activities depend on taxonomic knowledge for identifying endangered plants and managing populations and ecosystems. If we

are to adequately inventory plants and maintain information systems about them, there must be a commitment to precisely identify the gaps in our knowledge, training more taxonomists (domestically and internationally), providing support for taxonomic studies, and providing support to build and maintain facilities for specimens and information storage in which to carry out the studies.

Contents

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1. State of knowledge of plant diversity.

Our knowledge of the diversity and distribution of the estimated 265,000 kinds of species of plants that occur on Earth today is primarily based on the study of the millions of specimens of these plants that have accumulated and been permanently stored for study and reference in herbaria, which may be free-standing or, more commonly, parts of museums, botanical gardens, and universities. The study of these plants is the concern of the discipline of taxonomy.

Taxonomy, broadly defined, is the study of the characterization, naming, and classification of plants, animals, and microorganisms. Organisms are characterized descriptively by their physical attributes, including for example gross morphology, anatomy, chemistry, and cytology, as well as other attributes such as habitat preferences, flowering and fruiting behavior, physiology, genetics, and distribution. Information about the distribution of organisms is an important aspect of taxonomy, because a knowledge of where organisms occur is critical to so many other disciplines. They are named according to carefully developed and precise rules, which result in a system of nomenclature that assures easy, unambiguous communications about plants. The information gathered in characterizing organisms is used to classify them into schemes that reflect their relationships in various ways. Classifications may indicate evolutionary relationships and may aid in the identification of newly discovered organisms. The information that is contained in classifications is also useful in identifying newly collected specimens of previously known organisms. Thus taxonomy provides basic information about the nature and relationships of organisms and a means of naming them. A discussion of the diversity and distribution of wild plants is based on information accumulated by taxonomic studies of plants.

Modern study of plant diversity dates from the publication of Species Plantarum by the Swedish naturalist Carl Linnaeus in 1753. In this two volume work, Linnaeus described about 7,300 species of plants, representing the flora of the world as known to him at that time. He named each species, employing consistently for the first time the binomial system of nomenclature still used today. Under this system, each species of plant is assigned a name consisting of two Latin words, a generic name and a specific name. Thus the binomial for the white oak is _Quercus alba_. Linnaeus arranged the plants he described in a classification based primarily on sexual characteristics of plants.

In the two and a quarter centuries since the publications of Linnaeus's Species Plantarum, continued exploration and research has resulted in the discovery, description, and naming of thousands of new species of plants. New species are generally detected when studying newly collected specimens and comparing them with descriptions and specimens of previously known plants. New species continue to be discovered at the rate of several thousand each year. Occasionally such comparative studies uncover new species that are based on old, previously misidentified collections. In the course of these studies known species are restudied to gain a better understanding of their character, relationships, and potential uses. Based on our current knowledge, there are an estimated 265,000 species of plants alive on Earth today. About 15,000 of these are bryophytes, i.e. mosses and their relatives. The remaining 250,000 are vascular plants, those with internal conducting tissues, including the ferns, cone-bearing plants, and flowering plants. (In this discussion plants are circumscribed in the modern sense, which excludes the algae, 20-24,000 species, and fungi, about 100,000 species including the lichens, from the plant kingdom.) The remainder of this essay will be devoted to a discussion of vascular plants, those that are conspicuous in our day-to-day lives.

Our knowledge of plants varies tremendously. We know a great deal about a few species, particularly those that are important economically for one reason or another. This amounts to a few thousand species at the most, and mankind depends on only a few hundred species for most food, fuel, fiber, and ornament. Even among some of those plants most important to man, some fundamental facts remain obscure. For the vast majority of the quarter-million species of plants we know not much more than can be discerned from one or a few pressed, dried herbarium specimens. Basically we have a gross characterization of the plant, based on its morphology, have given it a binary name, and have crudely placed it in a broad scheme of classification.

Just as our level of understanding of individual species of plants varies tremendously, our knowledge of where they occur also varies greatly. However, it is clear that the majority of plant species occur in tropical regions of the world. One-third, or about 80,000, of the species of plants occur in temperate zones, and two-thirds, or about 160,000, occur in the tropics. The tropics of the New World are by far the richest with an estimated 90,000 species (Prance, 1977). Tropical Africa has about 30,000 species, with an additional 10,000 occurring on the island of Madagascar (Brenan, 1978). Tropical Asia, including tropical Australia, has about 35,000 species (Prance, 1977).

Taxonomic knowledge is basic to all conservation, since it provides information about what there is in the world and where it occurs. Without this information there would be no basis for answering the question, what is there to conserve. Present trends in taxonomy are refining species concepts beyond mere morphological characterization, especially in temperate areas; refining our knowledge of the distribution of plants; continuing the inventory of lesser known species, especially in tropical areas.

This knowledge of plants benefits plant conservation directly by providing information on the characteristics, distribution, and relatives of endangered plants. This information is essential to managing known populations, locating potential areas in which additional populations might occur, and suggesting areas for the introduction and establishment of new populations of endangered plants. Taxonomic knowledge of non-endangered plants benefits conservation of animals by providing information about their habitats, food sources, and their general environmental contexts, which are dominated entirely by plants in most areas. Without this information, management of known populations is impossible and discovery of the true extent (numbers, distribution) of populations is very difficult.

2. Gaps in knowledge of plant diversity

Two important gaps in our knowledge of plant diversity and distribution are 1) the lack of synthetic treatments of existing information and existing collections and 2) lack of collections, especially from tropical areas, on which to base such treatments. Multivolume, synthetic floras have been published for Europe (about 12,000 species; Tutin et al., 1964-1980) and the U.S.S.R. (about 17,500 species; Komarov et al., 1934-1964), and a flora of China (32,000 species; Institute of Botany, Academia Sinica, 1959-) is well underway, with 36 of a projected 80 volumes published to date.

The only north temperate area that does not have a modern flora is North America (15-18,000 species). Thus for the United States there is no single reference to which to turn to find out even the most basic information, such as the distribution of a particular species on a state-by-state basis, let alone identify a newly collected plant specimen from a particular region of the country. Nor is there any central data base that will answer many fundamental questions about the plants of the United States. The best that we can do is refer to several recently published checklists, mostly compiled from previously available information and reviewed by specialists in various families or geographical areas (see Shetler and Skog, 1978; Kartesz and Kartesz, 1980;

Soil Conservation Service, 1982a, 1982b). These provide regional distributional data, for example "North Central," and minimal information about the characterization of particular species, for example "perennial, native, epiphytic, woody vine" (see Shetler and Skog, 1978).

The situation with respect to tropical areas is much worse. Reasonably up-to-date floras have been published for a few, mostly rather small areas, for example Guatemala (Standley et al., 1946-1977), Panama (Woodson and Schery, 1943-1981), and long term projects, which will require decades to complete at current rates, are underway for some large areas, for example east tropical Africa (Turrill et al., 1952-   ), and Malesia (Steenis, 1948-   ). These efforts suffer from a general lack of adequate funding to support collecting in the areas under study.

Taxonomic knowledge depends on specimens for study, and without an adequate specimen base, published floras may be of little use in identifying plants. For example, publication of the Flora of Panama began in 1943 and ended in 1981. This flora is "monographic" in format, that is to say the species are treated with full descriptions, illustrations are provided for many and specimens studied are cited, with their locality and collector's name given. During most of the period from 1943 until the early 1960s, little collecting was carried on in Panama, and the volumes of the Flora of Panama published during that period were based on studies of collections made mostly during the period 1930-1945. After about 1965, exploration was resumed. Study of the newly collected specimens revealed that many previously unknown species existed in Panama and entirely new species were constantly being discovered. The published flora treats only about 6,200 of the estimated 8-10,000 species that occur in Panama (D'Arcy, 1980; D'Arcy and Correa, 1985). The earlier fascicles of the Flora, those published in the 1940s and 1950s are virtually useless, because they were based on a grossly inadequate sample of the flora. Exploration and study of the flora of Panama continues, and in the five years since the completion of the original publication an addition 2080 species have been catalogued as occurring in Panama, representing an increase of 40% over the flora known in 1981 (D'arcy, 1985).

Turning to the second gap, the lack of collections on which to base studies, the large land masses of the north temperate zone are fairly well collected, but the problem is particularly severe in tropical areas, where, of course, the greatest diversity of plants occurs. This lack of specimens means that we can only crudely estimate the number of species that occur in many regions of the globe, basing our estimates on the number of species known from areas with similar habitats and on the rates at which species are being newly reported from the areas under consideration. The South American country of Bolivia serves as an example. The area of Bolivia is about 1,098,000 square kilometers, three times the size of California. Bolivia lies entirely within the tropics. About 8,000 species of plants have been recorded from Bolivia, based on 90,000 collections. A reasonable estimate of the number of species occurring in Bolivia is 18,000 (Solomon, 1985). (About 5,000 species of plants are native to California, which has one of the best known and most diverse floras in the United States; see Munz, 1959.) We can predict something about which species will be discovered to occur in Bolivia, based on what we know about surrounding areas and plant distribution in general. But, we cannot really say much about the plants that occur in this enormous area. The situation for most of tropical South America is similar.

In order to arrive at an estimate of the effort required to fill these gaps in our knowledge about plants, we will make some broad assumptions, using the United States as a model. Based on a broadly based survey of the taxonomic community, Edwards, Davis, and Nevling (1985) estimate that there are about 1,900 to 2,400 plant taxonomists residing in the United States (Edwards, Davis, and Nevling, 1985). However, many of these taxonomists study plants from other parts of the world, and some study algae or fungi, which are not plants for the purposes of this essay. This reduces the number of taxonomists concerned with the flora of the United States, and a better approximation of the number is based on the membership of the American Society of Plant Taxonomists. The membership of this organization is made up of the most active workers in the country and consists of those primarily concerned with flowering plants and ferns, since the algae and fungi have their own special membership organizations. Estes (1985) lists about 990 members of the American Society of Plant Taxonomists, of which about 750 live in the United States. A study of the research interests of the membership indicates that about one-third of the members are interested primarily in non-U.S. floras or groups of plants or in specialized subdisciplines of taxonomy that do not contribute to basic, descriptive floristic knowledge. This reduces the number of workers concerned with the flora of the United States to about 500.

According to Shetler and Skog (1978) there are about 18,000 species of plants in the United States. Let us assume that the flora of the United States is reasonably well known - essentially all of the species have been discovered and collected enough for meaningful descriptions to be prepared and distributions to be mapped - and that the taxonomists currently studying the flora are engaged in research that will bring our knowledge of the flora to the state-of-the-art by the end of the century. Based on 500 taxonomists studying, collectively, 18,000 species, each is responsible for an average of 36 species. Extrapolating to a world scale and assuming that the flora of the rest of the world is as well known as the of the United States, which it manifestly is not, with 265,000 species of plants in the world, there should be a minimum of about 7400 taxonomists engaged in the study of these plants. A reasonable estimate of the number of taxonomists working in the world today is based on the index of personnel associated with the herbaria catalogued in the latest edition of Index Herbariorum (Holmgren, Keuken, and Schofield, 1981). There are about 1700 names listed,but again some are concerned with algae and lichens Thus the work force would have to be more than quadrupled, to at least 7,000, to adequately study the world's flora.

The model is complicated by the fact that most of the world's flora is not as well known as that of the United States. Some areas, particularly Europe are certainly better known, but most of the world is less well known. Two-thirds, about 190,000 species, of plants occur in the tropics, where as many as one-in-six species have yet to be discovered (National Research Council, 1980). The number of taxonomists should probably be doubled again, to perhaps 15,000, in order to provide enough manpower to carry out the very basic tasks of collecting and describing the plants of the lesser known regions.

Returning to the United States model, let us assume that the cost of supporting a taxonomist, including salary, benefits, support personnel, equipment, travel, and overhead, is on the order of $30,000 to $50,000 per annum. This means that the budget for plant taxonomy in the United States is on the order of $15-25 million dollars annually. Since most of our 500 or so taxonomists hold academic positions and devote only about one-third of their time to taxonomy, the rest going to teaching, administration and other academic duties, the actual amount of funding devoted to taxonomic studies is on the order of $5-8 million. Again extrapolating to an idealized world model, we should be spending something on the order of one-half billion dollars a year to support studies of the diversity and relationships of plants.

3. Storage and use of knowledge of plant diversity

Our knowledge of the diversity and distribution of plants is based primarily on herbarium specimens, samples of plants that are pressed, dried, labelled and permanently stored for reference and study. Although plants vary tremendously in size and shape, among other characteristics, they are generally easily pressed into specimens, which are glued or sewn to uniform size paper (about 12 by 18 inches). A label indicating the plant's name and detailing the place and date of collection, the collector's name, and other information that would otherwise by lost when the plant was removed from its habitat, for example the size of a tree, the soil type in which it was growing, names of associated species, uses to which the plant was put by indigenous people, is also attached to the paper. Clearly, certain kinds of plants, for example very large or very small ones, very bulky ones, require special preservation techniques and storage facilities, but the vast majority of plants are manageable using standard collecting, pressing, and storage techniques. This greatly simplifies the storage and handling of plants specimens in comparison with many animal groups. For the most part, herbarium specimens are permanent, many herbarium collections are well over 200 years old and, given protection from a few pests, are expected to last indefinitely.

Since the technique of preserving plants as herbarium specimens was developed in the late 1400s, millions of specimens stored in thousands of herbaria have accumulated. The locations of the collections are catalogued in a general way and published in the Index Herbariorum, which is updated regularly and contains a wealth of information concerning the locations and contents of collections. The latest edition (Holmgren, Keuken, and Schofield, 1981) lists about 1400 herbaria, containing about 50 million individual specimens. Some of these specimens are exact duplicates of one another, but they are housed in different collections, and others represent different specimens of the same species. Although it may appear that redundancy in collections is wasteful, it is important, because it protects against loss of specimens through natural or man-caused disasters: the herbarium in Berlin, which contained thousands of fundamentally important collections, was almost totally destroyed when it was accidentally bombed by Allied forces during World War II. Redundancy also means that botanists at different institutions can study specimens of the same species to determine variation in the case of several specimens of that species from different areas and collection times, or even many individuals or parts of individuals from the same population.

While herbarium specimens are the primary resource on which our knowledge of the diversity and distribution of plants is based, information about these topics, derived from the specimens, is, stored as printed material, for the most part.

Studies of diversity and distribution may be broken broadly into two groups: monographic studies and floristic studies, either of which may be published as separate books or as journal articles. Monographs are detailed studies of particular groups of plants. They may concern entire families, though they are usually restricted to a genus or even part of a genus of plants. Thus one may monograph the oak genus, Quercus, or a portion of it, for example Quercus subgenus Lepidobalanus, the white oak group. Monographic works contain information about the names, characterization, and classification of the group under study, and this information is usually supplemented with illustrations, maps depicting the plants' distributions, and discussions of each species. Dichotomous keys, which are used as a means of identifying unknown collections of plants, are also usually included in monographs. Finally, lists of the specimens on which the study was based are also usually included, so that users of the published information can return to the original material from which it was acquired for verification, clarification, or further study.

Floristic works, or floras, are concerned with all the plants that occur in a particular area. The area covered may be large or small, and it may be politically or naturally circumscribed. Examples of politically defined areas with published floras are the Carolinas (Radford et al., 1968) and California (Munz, 1959) and with naturally defined areas the British Isles (Clapham et al., 1962) and Jamaica (Adams, 1972). Published floras contain the same kinds of information that monographs contain, although generally less detail is provided. For example, descriptions may be shortened to include only diagnostic features and only commonly encountered alternate names or synonyms may be listed. Keys, illustrations, and distribution maps are commonly included in floras, however, since they are particularly useful in identification, which is the main use to which floras are put. The amount of information included in a floristic publication may be reduced to the bare minimum of listing the correct names of all the plants known from the area under consideration and nothing else. Such lists are called check lists (see for example Shelter and Skog, 1978).

Some idea of the mass of data that has accumulated about the diversity and distribution of plants may be gained from considering the number of entries in catalogues of the literature of taxonomic botany; the numbers are not precise. An analysis of the volumes of Index Kewensis (Jackson, 1893-1981), which attempts to catalogue the names assigned to seed plants and citations of their first place of publication, results in an estimated one million entries. Index Muscorum (Wijk et al., 1959, 1962, 1964, 1967, 1969), which catalogues the names for mosses, contains about 80,000 entries. There are an estimated 240,000 species of seed plants and about 12,000 species of mosses. This means that many species have been assigned more than one name and that infraspecific names, those of subspecies, varieties, and forms, exist under many species.

These names and other information dealing with plant diversity have been published since 1753 in hundreds of thousands of individual books and journal articles. A recent catalogue, Botanico-Periodicum-Huntianum (Lawrence, et al., 1968), lists over 12,000 individual titles of journals alone that contain articles about plants. Taxonomic Literature, second edition, which will be completed in seven massive volumes, will list 15-16,000 titles, mostly books, mostly published before 1939 (Stafleu and Cowan, 1976, 1979, 1981, 1983, 1985). Taxonomic Literature is not exhaustive: it is a catalogue of only the fundamentally important literature.

Taxonomic botany has only begun to move from storing information in printed form to storing it in electronic form.

Several data bases exist that catalogue names for selected groups of plants or plants from selected areas. These files of names are connected with files containing additional information about the plants, for example distributional data, chromosome numbers, chemical content, or uses. As individual data bases grow and as hardware and software become more generally available, it should be possible to start integrating information and to reduce some of the redundant data capturing that is now going on within the taxonomic botany community. In principle, each of the one million or so names that exist for plants need be stored only once and this information transferred in one way or another to multiple users who can then add supplemental data of the kinds just mentioned.

The uses to which information about plants are put are numerous, but they may be conveniently grouped under three general headings: basic botany, applied botany, and leisure and esthetic botany. Basic botanical studies are those that attempt to add to knowledge as part of our overall attempt to understand the world and universe in which we live. Such studies may involve more fundamental characterization of plants than those found in taxonomic studies, for example the physiology of a plant or some detail of the physiology of a plant; development of information about the interaction of one species of plant with other species of plants or animals. Applied botanical studies have the goal of exploiting plants in some way, for example as sources of food, fiber, fuel, or chemicals. Such studies may seek entirely new products, as in the case of deveveloping new drugs, or seek to improve an existing product, for example better yields from a particular crop. Finally, much use of botanical information is made by those who pursue knowledge of various kinds about plants as a leisure time activity. The importance of this use of botanical knowledge is reflected in the large number of gardening books and field guides to wildflowers and other plants, the organization of gardening or special interest floral clubs, the numbers of departments of horticulture in our universities, and the number of commercial concerns that deal with gardening and horticulture in various ways. These three kinds of uses to which botanical information is put overlap in varying degrees, but the fundamental thread that is interwoven through them is the need to have a name by which to refer to the plant under consideration, to know the nature, or characterization, of the plant, and to know how it is related to other plants. Characterization, nomenclature, and classification are the basic aspects of plant taxonomy.

Conservation is a area of activity that depends heavily on plant taxonomy, broadly overlapping the three kinds of uses mentioned above. Specific data banks, identification manuals, and monographic works are used by or developed by conservation agencies. Most information on endangered plants or environments is scattered in the enormous volume of literature mentioned earlier. If all this information were available, it would be enormously useful in developing conservation strategies. Currently, however, it is all too easy for inappropriate policies to be developed, based on insufficient information. This is particularly true in areas of high plant diversity, where ecosystems are incredibly complex and delicate. It is most essential to understand the diversity of plants and their interrelationships with other organisms in these areas, because their survival depends on maintenance of this ecological balance.

4. Constraints to improving knowledge of plant of diversity.

Progress in improving our knowledge of plant distribution and diversity is hampered by three factors: lack of sufficient manpower, lack of adequate physical plant, and the rapid disappearance of the very subjects of our study, plants.

A reasonable estimate of the number of properly trained people available in the world to deal with the problems of plant diversity is 1700, as mentioned above. Some of these taxonomists are generalists, dealing with broad floristic areas or taxonomic groups, but many specialize in local floras or detailed investigations of small taxonomic groups. Further, most of the people live in the temperate zones and work on temperate zone plants. Our greatest need for improved understanding of plants is in the tropics, where there are few well trained botanists. Another factor inhibiting progress in understanding plants is that most botanists are employed by universities or colleges and must spend most of their time teaching and otherwise dealing with academic affairs rather than pursuing botanical knowledge, as noted previously.

Generally a taxonomist requires about one technical support person to carry out routine functions associated with taxonomic studies. These duties include label preparation, plant mounting and filing, and shipping and receiving of specimens. Additional functions, related directly to research work, may include sorting specimens and scoring various character states on data capture sheets, maintaining living collections, and transcribing label data from study specimens.

Although herbarium specimens are easily prepared and, individually, do not require much storage space, the shear numbers needed to properly access distribution, both temporal and geographic, and variability mean that enormous storage facilities are needed to accommodate them. The numbers of specimens that should be collected to advance our knowledge of tropical plants to the current state of our knowledge of temperate plants is on the order of three or four times the number of plants currently stored in herbaria. Most herbaria are crowded with specimens today. Unless specimens are properly mounted and stored after they are collected, they are essentially useless as far as advancing botanical knowledge is concerned.

Given the known high rates of habitat destruction or degradation (National Research Council, 1980), especially in tropical regions, and the fact that much of this destruction cannot be halted, many plant species will become extinct before they are discovered, characterized, named, and classified. Many others will be known from only one or a few specimens, and little more than their gross characterization will be possible.

In order to overcome these constraints, funding must be made available to train more botanists, particularly in tropical botany, and to provide positions for them after training is complete. The pool of existing botanists would be employed in providing needed training, but the efforts of many could also be directed away from academic affairs and toward research on plant diversity. It is particularly important to train nationals in tropical areas, because of the lack of manpower there now, because of the economies of having in-country botanists studying with the local flora, and because of political pressures on foreign botanists working in tropical areas.

A well-trained, world-wide cadre of taxonomic botanists must be supported by additional trained technicians who would process specimens and data. Collections are worthless unless they are properly stored, and additional storage facilities, employing modern storage techniques, such as compactors, will be needed on a large scale. Because progress in understanding the taxonomy of plants depends so heavily on the knowledge that has already accumulated, consideration should be given to data banking much of this information on a massive scale and making it available to the botanical community. New data should be immediately electronically data banked, because this system of data storage can make information rapidly and widely available in a variety of forms.

The international network of institutional herbaria, free standing, academic, and governmental, is the logical medium through which the current constraints on advancing botanical knowledge should be overcome, because the collections and personnel, which form the basis for advancing knowledge, are in place in these herbaria. The botanical community has done a good job of establishing and maintaining a communications network, so that people with particular expertises and collections with particular strengths are easily located. Some botanists and some collections might logically be moved from place to place in order to strengthen existing resources and at he same time assume the long-term well being of specimens that might disappear through neglect. Based on existing networks and information, a large scale study of existing personnel and collections would provide the basis for planning the strengthening weak areas and perhaps de-emphasizing areas that currently may be overemphasized, in view of the crisis facing botanical inventory in the tropics.

5. Mechanisms to encourage improving knowledge of plant diversity

Because of the importance of the knowledge of plant diversity to the conservation of genetic diversity and resources and because of the inadequate size of the current pool of taxonomists, the inadequacy of facilities to continue to develop our knowledge of plant diversity, and the inadequacy of the current information storage and retrieval systems, there must be a national commitment to solve these problems. The botanical taxonomic community has performed a good job of monitoring its size and resources (Edwards, Davis, and Nevling, 1985; Holmgren, Keuken, and Schofield, 1981). With adequate, ongoing funding, a continually up-dated statement of national and world-wide priorities for increasing knowledge about the diversity and distribution of plants could be developed. However, without the assurance of funding to meet these needs, such an exercise is of little more than academic interest and would not produce the results for which it was designed, namely more knowledge about plants.

In a general way the areas that will require additional funding are 1) training: additional taxonomists, both U.S. and foreign; 2) storage facilities: construction and maintenance of facilities for the storage of specimens; 3) electronic data banks: of taxonomic information must be developed and maintained and their information made available to a variety of users at national and international levels; 4) research funding: without additional funding for research, the resources listed in items 1-3 cannot be properly utilized.

Over the past forty years the National Science Foundation has provided the most consistent funding for taxonomic botany, through its Systematic Biology and, more recently, Biological Research Resources Programs. Funding for these two programs, which supports, inadequately a present, points 3 and 4 above, should be increased. Additional programs within the Foundation specifically for "training in taxonomy" and "taxonomic information" should be established and provided with funds adequate to the needs of developing taxonomic knowledge.

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Contract Paper No. 4

New Technologies and the Enhancement of Plant Germ Plasm Diversity

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NEW TECHNOLOGIES AND THE ENHANCEMENT OF PLANT GERMPLASM DIVERSITY

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Abstract:

Growth and development of human society results in some degree of irretrievable loss of biological diversity. Recently, powerful new capabilities have been developed which enable one to genetically alter organisms with much greater speed and precision than before, and the possibility of actually creating desired new life forms lies within our reach. How and to what extent will these technologies contribute to the pool of plant genetic diversity? This paper summarized the state of the art with regard to a limited array of new technologies: micropropagation, cell culture, haploids, protoplasts and transformation. Of these, somaclonal variation, somatic hybridization (and in a limited sense), recombinant DNA/transformation appear to hold the greatest potential at present. However, they are currently all too inefficient, expensive, and

unpredictable to permit a meaningful contribution to biological diversity. To realize the full potential of these technologies for the augmentation of biological diversity, the following actions are highly recommended:

1. Fund research of plant biochemistry, development, and genetics at a meaningful and sustained level through existing agencies
2. Bring private and public biotechnology experts into the NPGS in an advisory capacity
3. Establish a grant program through USDA/ARS or CSRS to encourage research germane to plant genetic diversity.

I. Introduction

The rapid development and growth of human civilization has incurred an irretrievable loss of natural biological habitats, and associated reduction in total biological diversity. This biological diversity is presently considered of paramount importance to the engineering of new plants and animals to meet future demands of humanity and the changing environment. Systematic collection and maintenance of components of extant diversity is the only strategy used presently to stem the loss of these resources. The advent of so-called new "biotechnology", namely the ability to manipulate cells and genes in the test tube, has prompted speculations as to where, when, and how these technologies can be made useful. This paper constitutes an attempt to assess as completely as possible the potential impact of biotechnology on biological diversity and its processing into finished products.

II. Emerging Technologies

A. Micropropagation

This constitutes a relatively recent outgrowth from the more general area referred to as asexual propagation. This is quite simply the use of somatic tissues of a plant to obtain two or more new identical plants. The practice probably started in many places independently, for example the propagation of olive, fig, or grape by simply thrusting cut stems into soil (Hartmann and Kester, 1983). Later, grafting and budding techniques were developed which greatly expanded the range of applicability. Many important plant species produce specialized asexual propagules such as tubers (potato, Jerusalem artichoke), bulb (Lilaceae), bulbils (garlic), and rhizomes (banana), which are typically used to establish crop stands. With respect to the natural generation of biological diversity, asexual reproduction would be considered a short term evolutionary strategy, culminating in a progressive erosion of genetic variability to the most successful and asexually fecund genotypes. The impact of asexual propagation as an agricultural tool will be addressed later.

Micropropagation, sometimes called plant cloning, is simply asexual propagation based on small amounts of tissue or cells and usually conducted in an aseptic environment with the assistance of artificial support media. At one extreme, this involves excision of dormant meristems from various parts of a plant, placing onto a defined support medium to break dormancy and permit the meristem to develop into a shoot, to induce roots to develop on a separate medium, and reacclimatizemate the resulting new plant to the soil and the normal open environment. At the other extreme it involves an explant tissue, not necessarily a meristem, placed on a medium which stimulates cells to proliferate individually. In certain species, most notably in the Umbelliferae, proliferating cells can be induced to undergo a process, superficially analogous to embryo development, referred to as somatic embryogenesis. Such somatic embryos have been successfully regenerated into whole plants.

Micropropagation as a concept and, eventually, a bonafide tool had its beginnings in the work of Haberlandt (1902), who first published on the possibility of *in vitro* (in glass) culture of tissues and organs. In 1939, culture of unorganized plant tissues (callus) for undefinite periods was reported (summarized in White 1963). The most important event by far was the discovery, by F. Skoog and associates that by altering the levels of the plant hormones auxin and cytokinin in the medium, shoots and/or roots could be consistently induced de novo from tobacco callus tissue (Skoog and Miller 1957). It was not long thereafter that the phenomenon of somatic embryogenesis was discovered in carrot (Reinert 1959, Steward et al 1964).

Despite these exciting advances, micropropagation, as with other cell or tissue culture related technologies to be addressed further on, has remained highly empirical. There still exists an enormous void of knowledge with respect to the physical, molecular, macromolecular, sub-cellular, cellular, and supercellular mechanisms contributing to developmental processes, such as the transformation of unorganized cells or tissues into shoot meristems or embryos. In the meantime, scientists are reduced to "pushing buttons" on the outside and watching what happens. From the beginning, it has been readily apparent that generalized tissue culture/micropropagation approaches developed for tobacco, carrot, and *Datura* vary tremendously in degree of applicability to other plant species. To some extent, one is forced to return to the drawing board every time. There are some general trends (with many exceptions): monocots, grain legumes, and woody perennials are recalcitrant whereas herbaceous dicots tend to yield more readily.

Micropropagation has been used to produce plants commercially in the ornamental industry for several years. This implies a distinct advantage in the efficiency of production over conventional approaches, especially since expensive facilities, sophisticated equipment and trained personnel are required. The prevailing attitude in the ornamental industry is presently conservative, yielding the conceptual attractiveness of micropropagation to the realities of business. The state of the art of micropropagation as a tool is limited by the "push button" state of the technology and practical aspects associated with handling.

With respect to genetic diversity, micropropagation in theory can have, as in nature, only a negative impact on genetic variability because only the desired components of an array are selected and perpetuated. In a genetic sense, non-additive sources of variation are likely to be exaggerated when individual plants are selected and asexually propagated.

Micropropagation will likely affect breeding methodologies in certain crops where significant problems can be alleviated or advances achieved. For example, individual plant hybrids embody a breeding concept which is a striking departure from breeding methods described in textbooks. For now, this approach will have very limited application, specifically to high cash value vegetable crops where production costs can be justified (Lawrence and Hill 1982, 1983). In its simplest sense micropropagation, or plant cloning, can be a powerful cog within a conventional breeding program, where exact plant "copies" would be useful. For example, where the determination of phenotype is destructive, the availability of clonal plants for the assay obviates the need for more inefficient, ineffective progeny tests. If it is difficult to propagate plant material sexually, such as with self-incompatible inbreds in _Brassicas_ or gynoecious cucurbits, highly efficient cloning protocols may offer an alternative. Genetic male sterility is known in many crop species, but is rarely used for commercial hybid seed production primarily due to the high costs associated with culling fertile plants from female rows. If one could identify sterile plants prior to flowering and clone them to numbers sufficient for seed production, this problem could be eliminated.

The possibility of establishing crops directly from clones (Lawrence 1981) is exciting, but potentially dangerous from the standpoint of genetic variability. As this technology progresses, it will be important to develop in parallel means to avoid genetic vulnerability, for example to build genetic diversity into phenotypically uniform clonal populations.

It is likely that this technology will make steady advances in the absence of any changes. The main limitation standing in the way of realized potential is simply our lack of understanding of development. It is widely presumed that this knowledge will surely lead to a roadmap of success in achieving tissue and cell-based cloning systems across a broad spectrum of crop species. As with other forms of biotechnology to be addressed later, continued and expanding fundamental research in this area will be critical to solving this challenging set of problems.

Button-pushing will continue to deliver small advances which will accumulate significantly over time. We are at a point now in some crops where the technology has become commercially feasible (see discussion above). Because of the empirical nature of the research, much or most of it is conducted behind closed doors in the private sector. Protocol originators tend to covet their "tricks" and communication of meaningful explicit procedural details is rare. Inevitably, this tends to isolate sets of observations which could add up to significant general trends.

B. Cell Culture

This term implies a process consisting of the isolation of tissues or cells of an organism, inducing them to grow in a prolonged state more or less as single cells, and the possibility of regenerating them back into plants. Applications of this process in micropropagation have been described earlier. This section will focus on the antithesis of cloning, the efficient generation and selection of new variability.

some of the earliest reports of regeneration from cultured plant cells came hand in hand with comments about the similarity between plant cell and microbial cultures (Melchers and Bergman 1959). Carlson (1972) advanced compelling arguments for the application of bacterial genetic approaches in higher plants by virtue of cell culture. Specifically, a rapid burst of information leading to the elucidation of biochemical pathways and gene expression had resulted in the 1950's and 60's from the accumulation, complementing, and assorting of bacterial mutants. Some mutants exhibited potentially useful properties such as resistance to antimetabolites (so-called selectable markers) or those overproducing desirable or even valuable compounds. This was all possible in bacteria as opposed to higher organisms because rare mutational events could be effectively recovered from large (10^6 to 10^8 organisms per ml) haploid populations. Carlson (1972) reiterated the similarity of a plant cell suspension to that of a bacterium, and expounded on the possibility of selecting rare mutants of potential agricultural significance in a similar fashion, e.g. salt tolerance, resistance to pathotoxins, and feedback insensitivity of biosynthetic enzymes leading to the accumulation of desired end products. In 1973, Carlson reported on the successful isolation of a tobacco cell line resistant to methionine sulfoximide, an analogue of the bacterial wildfire toxin. Later, Maliga et al (1975) isolated a tobacco cell line resistant to the antibiotic streptomycin. Plants were regenerated and subjected to genetic studies. Resistance was found to be transmitted to progeny and inherited materially. Technology appeared to be standing on the brink of a veritable revolution, characterized by plants exhibiting new valuable characteristics regenerated from cell cultures screened under the appropriate conditions. More than ten years later, it is safe to say that we have not undergone a revolution of any substantial proportions for reasons which will be addressed later. However, some notable successes have been achieved, such as salt tolerance, disease resistance, amino acid overproduction, and a limited spectrum of additional antibiotics or antimetabolites (see recent reviews by Maliga (1984) and Meredith (1984)).

The occurrence of mutants in plant cell cultures implies underlying variation, an interesting subject in and of itself. Bacteria and fungi proliferate on artificial media, usually supplemented only with simple nutrients and a carbon source, more or less as they would in nature. The natural mutation rate in such cultures is normally b[illegible] on the order of 10^{-6}. Mutagens such as DNA-insulting chemicals, X-rays, or ultraviolet light are used rather routinely to increase the mutation rate by two to four orders of magnitude. Following this example, early attempts to obtain specific mutants included artificial mutagenesis. Later, Chaleff and Parsons (1978) showed that mutants could be selected rather effectively from non-mutagenized cultures. A further implication of this report was the natural mutation rates may be inordinately high in plant cell cultures. It had been known since the early reports of Mitra and Steward (1961) that cultures can exhibit considerable variation with respect to chromosome number, and this observation has since been elevated to the status of generality (Bayliss 1980, Orton 1984). More recently, numerous reports have appeared wherein high frequencies of mutations (or stable variants) appear in populations of plants regenerated from non-mutagenized, non-selected cultures (summarized in Orton 1984). Larkin and Scowcroft (1981) advanced the notion of using this variation in the context of breeding, and named it "somaclonal variation." Perhaps surprisingly, this undirected approach has already yielded potentially valuable mutations such as disease resistance, male sterility, loss of abscission tissues (preventing fruit drop or damage during shipping), seedling vigor, and wheat kernel pigment changes (Larkin et al 1983).

Getting desired mutations in higher plants is at best an imprecise and improbable venture regardless of the route taken. Most significant characters would appear to be hopelessly inaccessable due to the large numbers of interacting genes involved in their determination. Devising schemes to directionally target only the genes involved, for example with population yield, let alone in vitro screening approaches is presently beyond our capabilities. Simply inherited traits, such as those which have already been successfully isolated (see above discussion) are somewhat more within our reach, but not without significant problems. In searching for a hypothetical new trait in a cell or plant population exhibiting natural or induced variation, one must first presume the character to be obtainable within the context of existing genetic and metabolic, and structural machinery. If employing a screening procedure on cells the problem arises of translating cellular into whole-plant phenotypes. Variant colonies arising with or without enrichment may, for unknown reasons, simply refuse to regenerate, or give rise to abnormal or sterile regenerates, signifying the end of the road for sexually propagated crops. The cell-plant translation and regeneration problem have been circumvented by performing chemical screening on mutagenized barley seedling populations, resulting in the successful isolation of amino acid overproducing mutants (Miflin et al 1983). However, the disadvantages of scale of this work as compared to the hypothetically perfect cell culture system are striking.

Somaclonal variation is on the surface an extremely attractive alternative to germplasm screening to the breeder. In its most powerful projected role, one simply uses it to correct deficiencies or obtain incremental improvements in otherwise superior lines. This as compared to finding genes in divergent germplasm and necessary recurrent backcrossing to the superior line. Such situations have been reported recently in wheat and tomato. In the former, the cultivar "Yaqui 50E", a hard red winter type, was cultured and regenerated. Among the array of mutations were white-kernel types exhibiting simple inheritance patterns (white are preferable to red kernels in Australia; Larkin et al 1983). In tomato, Evans and Sharp (1983) regenerated plants from leaf callus tissue of the commercial processing cultivar "UC82B". Among the useful mutations recovered were both simply inherited (jointless pedicels; male sterility) and quantitatively inherited (soluble solids; Evans et al 1984) forms.

Somaclonal variation is potentially an even more powerful breeding tool in asexually propagated crops such as potato, sugarcane, banana, herbaceous ornamentals and a broad array of woody perennials. In such crops, cultivar development is hindered by the inaccessability to sex, polyploidy, of tissue chimeras, not to mention long generation times. As a consequence, variant selection schemes predicated on the spontaneous or induced appearance of "sports" are usually preferable to the exploitation (where possible) of sexual systems. Variants are simply recognized and propagated. Somaclonal variation is operationally identical. Mechanistically, we do not presently understand enough about the process by which variation is generated to conclude whether somaclonal variation is qualitatively different from that which is naturally occurring during asexual propagation.

Somaclonal variation has been reported extensively in potato (Shepard et al 1980) and sugarcane (Heinz et all 1977). In potato, leaf mesophyll protoplasts from the cultivar 'Russet Burbank' were cultured and regenerated. Almost 25% of the regenerated plants exhibited striking phenotypic or physiological (e.g. disease resistance) departures from 'Russet Burbank'. According to Gary Secor (1984), however, almost all, if not all, of this variation seems to be unstable during the course of propagation by tuber.

Plants regenerated from callus cultures of sugarcane also exhibit high levels of morphological variation and in response to pathogens (Herring et al 1977). At least some of these variants appear to be unstable. Lutz (1985) speculates that yield increases may be due to the purging of cryptic virus', which subsequently re-enter the clone and depress yield. One would predict based on simple inheritance patterns observed for variants in seed propagated crops that at least some of this variation should be stable. Since many of the mutations are recessive, they may be perpetually masked in clones.

It is not presently possible to speculate meaningfully on the overall lasting impact of plant cell culture technology on crop improvement. It is safe to say that it will, to some degree, enhance diversity by adding new allelic forms to the gene pools of crop species. As we understand more about economically significant traits such that in vitro selection systems can be devised, new avenues to enrich for such characters will certainly result. Somaclonal variation has been in some cases unstable in sugarcane and in potato, and mutations affecting flower color recovered from alfalfa cultures show high reversion frequencies (Bingham 1984). As desirable somaclonal variants emerge from culture dishes, it will be incumbent upon breeders to subject them to highly rigorous field testing especially from the standpoint of instability and negative pleiotrophic effects.

Somaclonal variation for the enhancement of genetic diversity in plants holds great potential also because it can under some circumstances be very efficiently generated. More research is needed to understand the molecular basis of somaclonal variation such that we can more effectively measure it, contrast it with naturally-occurring genetic diversity, and direct its manifestations in a predictable fashion.

For the present, it is more comfortable, and probably appropriate, for the plant breeder to opt for the world of whole plants grown in glasshouses and fields as opposed to the unknowns of cell culture approaches. Rather, these new tools are more likely to be stocked in the chest alongside mutagenic and mitogenic agents and wide hybridization as "desperation" alternatives to crop improvement dilemmas. Research into the molecular events which underlie the determination of economic traits such as morphology and photosynthate partitioning will presumably lead to the formulation of methods to improve precision and efficiency of selection. Likewise, research into processes by which mutations occur or are induced in somatic tissues will, it is hoped, provide insights into its control and targeting. These are tremendously challenging problems which will require patient, consistent, and realistic support before solutions are apparent. It should not be difficult to identify and support individuals in the public sector to conduct research on the molecular biology of phenogenesis. Conversely, somaclonal variation has evolved into a popular tool in the plant biotechnology sector, but very few fundamental public programs are addressing the problem. In the experience of the author, communications among the various entities conducting mechanistic and applied research on somaclonal variation are very poor, even to the point that attempts to resolve standardized nomenclature systems have failed.

C. Haploids

Induced euploidy changes have been intriguing and confounding to plant breeders since the discovery of the mitogenic effects of colchicine. With this magical compound, one can readily convert diploids directly, or indirectly into an array of polyploids. Comparative studies of diploids and polyploids showed them to be generally of larger proportions, albeit of lower fecundity (Dewey 1980). Fertility was found to be exceedingly low in triploids and other odd multiples, as predicted from the process of gametogenesis. Breeding systems predicated on tapping into higher vegetative and partitioned yield and seedless fruits offered by polyploidy were spawned. Decades of frustrated attempts to validify these approaches have fallen far short of early speculations. Dewey (1980) has published an excellent recent review of where induced polyploidy has been, and where it is likely to contribute in the future. Successful commercial entities resulting from this approach are presently limited to forage grasses and legumes, and novelty seedless fruits (e.g. watermelons, oranges).

At the other end of the spectrum lies the possibility of obtaining haploids directly from diploids or polyploids. This possibility became apparent first as a result of the pioneering research of Guha and Maheshwari (1964) and Nitsch and Nitsch (1969) who successfully regenerated haploid plants from cultured anthers of Datura innoxia and Nicotiana tabacum. Such haploid plants must have arisen from microsporcytes, following the reduction division of meiosis, whose developmental pathway had been diverted from pollen to somatic tissue formation. It was initially presumed that empirical research would eventually yield successes in obtaining haploids from a broad array of plant species. In 1969, Kao and Kasha reported the remarkable finding that haploid barley plants could be obtained from zygotic embryos resulting from the interspecific cross of diploid barley to a wild weedy relative, Hordeum bulbosum. Later cytogenetic studies revealed that chromosomes of the latter parent were "eliminated" from somatic cells of the embryo over the first two weeks of development (Kasha

1974). Since then, the phenomenon of chromosome elimination has been found to be rather common among interspecific hybrid combination in the Gramineae subfamily Hordeae, but rather infrequent elsewhere in the Plant Kingdom.

In the early 1970's with anther/microspore culture and chromosome elimination emerging as powerful technologies for the large-scale production of haploid plants, utility for improving the efficiency of plant breeding was realized. Haploids are entities from which "instant inbreds" may be obtained by simply doubling the chromosome number to the diploid level. Predominant breeding schemes, e.g. pedigree and F_1 hybrid methods, call for inbreeding for line or parent development. Inbreeding is almost universally accomplished by recurrent self-pollination. Starting from heterozygosity, allele fixation occurs at the rate of 50% per generation per locus, and functional inbreds are generally thought to require at least six cycles of recurrent self-pollination. In certain crops, physiological or morphological barriers make self-pollination difficult or impossible, for example self-incompatibility (Crucifers) or dioecy (spinach). In these cases, induced haploidy carries an obvious additional incentive. An International Symposium was held in Guelph, Ontario, Canada in 1974 devoted to the subject, seeming to usher in a new era of advances.

As with other approaches, the revolution has fallen short of expectations, but has brought forth some of the most notable economic successes within the realm of plant biotechnology. Several successful barley varieties have emerged from independent programs utilizing the bulbosum crossing method, which now stands as a common tool worldwide in this crop. Anther culture techniques have been developed for wheat and rice, primarily in China, where they are now in widespread use. Cultivars produced using these methods now comprise a significant area in China. In Cruciferous crops (e.g. cabbage, mustard, oilseed rape, etc.), highly effective anther and microspore culture methods have been described (Keller et al 1975). These are only now beginning to take hold in the breeding of these species, and notable contributions should be evident by the late 1980's. Broadening the base of crop species from which haploids could be reliably obtained has been, unfortunately, quite frustrating. Anther/pollen culture, ovule culture, or wide crossing have likely been attempted more or less systematically with a large number of crop species beyond those listed above without notable successes. In tobacco, anther culture has been successful experimentally but resulting plants appear to contain genetic lesions which render them commercially unattractive.

As discussed previously, the value of haploids lies in the ability to produce an array of homozygotes from a polyheterozygte, which is an operation very common in breeding both cross and self-pollinated crops. The technique theoretically will bring little in the way of actual new germplasm onto the scene, only a way to deal with it more efficiently. In fact, the degree of realized genetic variability released into inbreds via haploids as compared with selfing or recurrent selection is reduced owing to a reduction in the cycles of recombination and, in some cases, the small sample used. Fewer rare recombination gametes between tightly linked loci will be recovered. It should be pointed out that the method has no theoretical impact on total potential genetic variability when adequate sample sizes are involved.

This technology, where effective, will revolutionize the process of crop improvement. What are the factors which presently render it possible on such a limited scale? Once again, one can point to a conspicuous lack of understanding of underlying processes, both in microspore culture and genome elimination from interspecific hybrids. As with other cell culture based technologies, significant progress will only be possible through sustained empirical and basic studies, the ultimate outcome of which are not at present predictable.

D. Protoplast Manipulation

Until relatively recently, it has not been possible to apply capabilities developed for bacterial or cultured animal cells to plants, for example direct DNA uptake or cell fusion. This is due to the presence in all high plants of a rigid polysaccharide cell wall. In 1962, Edward Cocking first successfully liberated significant numbers of naked cells, called protoplasts, from this encasement; this was first done by simply slicing leaf tissue. Subsequently, the use of powerful cell wall degrading enzymes purified out of fungi and gastropods was developed, and this remains the method of choice. It is now possible to routinely isolate millions of protoplasts from one or more types of tissue of a broad array of plant species. Protoplasts offer a number of intriguing possibilities for genetic modification.

1. Populations of clonally derived plants exhibiting spontaneous somaclonal variation.

2. Populations of clonally derived plants transformed with naked DNA or engineered Agrobacterium sp.

3. Fusion of protoplasts to obtain novel polyploids or hybrids.

4. Rapid transfer of traits encoded in the genomes of cytoplasmic organelles.

The first subject was covered in some detail in a previous section, and the second will be discussed later. Somaclonal variation and transformation may be accomplished without protoplasts, but they are paramount to plant cell fusion. The first successful plant protoplast fusion was reported by Carlson et al (1972), wherein leaf mesophyll tissues of Nicotiana glauca and N. langsdorfii were digested with cellulytic and pectolytic enzymes and fused under conditions of high calcium and pH. While it heralded an exciting possibility, this experiment did not actually yield a hybrid which could not have been obtained by sexual hybridization. Shortly thereafter, Kao and Michayluk (1974) introduced the use of polyethylene glycol (PEG) as a powerful fusigen, and this has remained the most popular method to the present. Numerous researchers are experimenting with electric fields as a method of inducing fusion, but it is presently too early to speculate what role this approach will ultimately play.

Like unconstrained sexual hybridization, fusion provides the possibility of bridging all existing gene pools. The obvious question, alluded to above, was whether sexually "impossible" hybrids could be obtained via fusion. Melchers et al (1978) finally achieved this with the curious tomato + potato somatic hybrid. Ironically, close examination of this potentially exciting hybrid revealed its potential role in breeding. As with Raphanobrassica, a hybrid between radish an cabbage produced sexually some 50 years ealier, the hybrid seemed to exhibit the least desirable characters from each of the parents. Moreover, it was completely sterile, and thus useless as a breeding intermediate. It appeared that the lessons learned earlier in the century, wherein it was concluded that all but the very closest of phylogenetic relative cannot be sexually hybridized due to isolating mechanisms. It was always felt, however, that incompatibility was a function of gamete-flower interaction. Since cell fusion and subsequent culturing and regeneration overrides this level of interaction, any hybrid should be possible. Indeed, stranger combinations tried, and some of these heterokaryons survived to make colonies, such as soybeans + tobacco (Kao 1978). It would appear based on the evidence at hand that somatic hybridization also has phylogenetic limitations in a gene-bridging sense; fusing an onion with a sunflower is more likely to yield genetic suicide than a duel purpose crop plant.

Attempts to obtain somatic hybrids between sexually incompatible species declined rapidly after the successful tomato + potato demonstration. However, research on protolast culture and new exploration of the concept of limited genome or subcellular transfers has been quite active up to the present primarily in Europe (Cocking et al in England; Pelletier, Vedel, Belliard et al in France; Schieder et al in

FRG; Maliga, Dudits et al in Hungary and Galun in Israel). As a result of the work of these groups and others, new exciting possibilities for protoplast fusion are emerging which are much more tenable than the earlier hybridization concepts. On the one hand, Schieder (1984) and co-workers have accomplished interspecific gene transfer by fusing recipient protoplasts with irradiated donor protoplasts. Presumably, the high radiation doses effectively "pulverize" the donor genome, but some donor sequences make their way into the recipient genome by somatic recombination or simple translocation. In their demonstration experiments, this group devised a clever enrichment procedure such that rare specific gene transfer events could be selected and readily confirmed. Intuitively, this would appear to accomplish in large part what somatic hybridization had originally hoped to in that genes from sexually incompatible species could be accessed without potential problems associated with genome incompatibility.

Yet another exciting alternative lies in the transfer of cytoplasmic traits via protoplast intermediates. Kung et al (1975) first showed that a maternally inherited trait, electrophoretic mobility of fraction I protein "sorted out" in clones derived from fusions, indicating that heterogeneous organelle mixtures ("cybrids") are unstable. In later studies involving fusions of tobacco differentiated by mtDNA restriction patterns, a common thread was observed: a broad array of different mtDNA patterns, ranging from the two original parents, to what appeared to be recombinants between the two (Belliard et al 1979). However, with respect to cpDNA, only the two parental patterns were recovered. The phenomenon appear to hold generally as they have been reproduced in Brassica (Pelletier et al 1983) and Petunia (Boeshore et al 1983, Cocking, 1984). Using a series of selectable and visual markers linked in cis and trans, Maliga (1985) has recently demonstrated convincingly that recombination can also occur between chloroplast genomes in a cybrid, albeit at much reduced rates as compared to mitochondria (as judged by the plethora of restriction patterns). It appears virtually certain that limited cytoplasmic and nuclear gene transfers obtained via fused protoplast intermediates will contribute novel and potentially useful genetic combinations to the plant breeder and overall pool of variation.

The ability to manipulate protoplast intermediates is a potentially powerful genetic tool for all plant species. Unfortunately, as with other cell-based technologies addressed earlier, this capability has been demonstrated only within a limited array, including primarily Solanaceous (tobacco, potato, petunia, tomato, Datura innoxia), Cruciferous (cabbage, oilseed rape), and Umbelliferous (carrot) species. Conspicuously absent are major grain species, such as soybeans, corn, wheat, and rice, woody perennials, and just about everything else. This is at least in part due to lack of trying, but this is certainly not the case for the species listed where thousands of scientist hours have failed to produce significant gains. In species for which complete protoplast to plant protocols have been attempted, but not successfully, the block usually manifests itself at stage 2; getting isolated protoplasts to divide. More rarely, divisions will begin but are not sustainable. Even more rarely, colonies and perpetual callus tissue may be obtained, but cannot be regenerated, such as with soybeans.

Because existing protocols were derived in a highly empirical fashion, they are sometimes replete with operations of questionable value, and may demand some of such subtlety and precision that they defy description. It is a common complaint that published protoplast culture protocols are not strictly reproducible. Scientists in both the private and public sectors are generally very protective of their specific methods, and a lack of communication may be a contributing factor to the lack of insights--common threads from contrasting systems may illuminate general problems and suggest solutions. Ultimately, the same lines of basic developmental research described in previous sections will impact on the regeneration problem, but may not go far toward solving the inability to induce or sustain cell divisions. That problem is as formidable as the whole of biology itself, and much patience in supporting basic research in general will be necessary before we are able to say with any certainty why a given cell is or is not dividing.

From the standpoint of overall biological diversity, protoplasts offer tremendous potential as tools for enhancement, albeit on a limited scale. They may or may not be desirable from the standpoint of recovering somaclonal variation as contrasted to technically less challenging approaches. The potential exists to bridge a wide range of nuclear gene pools by direct hybridization or, more likely, by limited genome transfer. New possibilities for nuclear/plastid/

mitochondrial combinations have emerged from research on the production and culture cybrids. A further consequence of this work is the finding that organelle genomes can undergo recombinational processes, giving rise to cytoplasmic genotypes otherwise unobtainable. Finally, protoplasts are necessary intermediates in getting desired molecules (proteins, DNA sequences) and supramolecular complexes into plant cells, as will be discussed in the following section. Meaningful and sustained support of basic plant research at the molecular and cellular level are urged to speed the development of systems in recalcitrant species.

E. Transformation

Ever since O.C. Avery and his colleagues first demonstrated in 1944 that bacterial cells can be induced to take up, integrate, and express exogenous DNA, extensions into eukaryotic systems have been pursued. This process, called transformation, is today the most powerful aspect of molecular engineering. Even where vectors are used to shuttle sequences into eukaryotic cells, transformation of bacterial cells is used extensively during the engineering of constructs. It was inevitable given the parallels between eukaryotic cell cultures and microbial populations that the possibility of transformation would enter the picture (e.g. Carlson 1972). Gresshoff (1975) first claimed success in transforming plant protoplasts with the demonstration that protoplast from white-flowered tobacco plants exposed to DNA from red-flowered genotypes infrequently gave rise to red-flowered regenerates. The authors referred to the phenomenon as "transgenosis". Others had difficulty repeating the work, and the concept languished for a period. By adapting the biochemical approach of feeding labeled precursors to elucidate pathways, a number of studies showed that naked DNA exposed to protoplasts is rapidly degraded. Most of this degradation occurred exogenously, but at least some of the DNA appeared to be entering the cells before disappearing into the nucleotide salvage pathways. These studies provided no evidence of integration in to the host genome, although resolution was poor. Parallel research in mammalian cell culture resulted in success in the late 1970's, thus providing some precedent for hopes of achieving transformation in plants. These experiments utilized selectable phenotypic antibiotic resistance markers, thus permitting the detection of very rare events. As it turns out, selectable antibiotic resistance markers were to play a substantial role in the development of transformation protocols in plants as well.

Since most of the DNA was being degraded exogenously, it was appropriate to explore ways of protecting it during the process of uptake. The most popular of explored approaches was to encapsulate the DNA in spherical bodies delineated by lipid monolayers or bilayers: so-called liposomes. Liposomes were easily generated, and potentially fusable with the lipid bilayer of plant protoplasts. Much research on liposomes using the "brute force" tracer screening approach could find only intracellular degradation of encapsulated DNA (Fraley and Horsch 1984). It was not until a bacterial gene (neomycin phosphotransferase from Tn5) conferring resistance to the antibiotic kanamycin was encapsulated that success in this approach was actually demonstrated (Caboche 1984, Potrykus, 1985).

In the early 1970's, the crown gall disease began to attract much interest and, ultimately, experimental activity. Pathogenic strains of the gram-negative bacterium Agrobacterium tumefaciens, when in physical contact with wounded plant tissues, induce the formation of a gall, or tumor. These tumors embody an interesting case of genetic parasitism: the plant cells are induced to synthesize and secrete peculiar amino acids called opines. The bacteria have all of the biochemical machinery to break these compounds down and use them for energy and resynthesis. Two main groups, or strains of pathogenic A. tumefaciens were recognized early on: those that caused the host cell to synthesize octopine or nopaline as the predominant opines. A broad spectrum of dicots form galls when wounds are exposed to pathogenic A. tumefaciens strains, but no monocots (including major cereals). Braun and White (1943) first determined that the tumorous phenotype persisted indefinitely after removal of the pathogen with antibiotics. Thus, some tumor-inducing (Ti) "principle" was transferred from the bacterium to the plant cell. The realization that the Ti principle could be integrated and expressed DNA heightened interest in the system as a potential method of transformation. From that point to the present, the story reads like a biological fairy tale. Chilton et al (1978) made the discovery that a small piece (T-DNA) of the large Ti plasmid in pathogenic A. tumefaciens was present in the nuclear genome of transformed cells. Advances in the fundamental molecular biology of crown gall and efforts to manipulate the system to achieve transformation came rapidly thereafter. Methods to move sequences involved in transformation into E. coli for ease of manipulation were important to later advances. Restriction mapping revealed common and variable patterns when comparing the T-DNAs of octopine and nopaline strains, but no apparent wealth of advantages or dirge of disadvantages seemed associated with either type, and different groups have used both successfully. Fraley et al (1983) finally achieved the first successful transformation using the NPT gene spliced into T-DNA. Ultimately, they demonstrated that transformed cells were resistant to the antibiotic and that NPT sequences were expressed. Subsequently, they successfully regenerated transformed cells and performed genetic studies that showed Kan^R to segregate in a Mendelian fashion. The NPT gene has now been used extensively to assist in the transformation process, serving as a selectable marker inserted into constructs containing genes of interest. Shortly thereafter, Murai et al (1983) reported the successful expression of a *Phaseolus vulgaris* (common bean) gene in sunflower (*Helianthus annuus*).

The large Ti plasmid carries, in addition to maintenance functions, two distinct regions critical to transformation: vir and T-DNA, the former of which is necessary for transformation but remains in the bacterium. Once transferred into the host genome, the tumorous phenotype results as a consequence of the gene products of the so-called Tumor morphology genes located in the left (T_L) region of T-DNA: TMS, TMR, and TML. Inactivation of these genes results, respectively, in tumors exhibiting shoots, roots, or large size. A number of workers (cited in Nester et al 1984) have used these mutants to show that auxin/cytokinin balances were profoundly affected, suggesting that TMS and TMR somehow interact with the synthetic or catalytic machinery of the host to induce the tumorous state hormonally, much as the tissue culturist does with artificial media.

One bottleneck of the system has been the difficulty in moving the large (approximately 200 kb) Ti plasmid between bacteria. This problem was in large part overcome by developing a binary system wherein the transforming bacterium contained an altered Ti plasmid carrying maintenance and vir functions, and a second, termed micro Ti, carrying T-DNA and replication functions. Since micro Ti plasmids are smaller (about 2 kb), they are much more easily handled and mobilized. Vir and T-DNA complement well in trans to reconstruct a virulent A. tumefaciens (Hoekema et al 1983).

A substantial amount of developmental research has been accomplished with A. rhizogenes, a close phylogenetic relative of A. tumefaciens which incites a lesion known as "hairy root". The molecular bases of hairy root are substantially similar to those of crown gall: pathogenic strains all contain a large plasmid, Ri, which is clearly different from Ti, but contains much sequence homology, including T-DNA. Tepfer (1984) has pointed out that tumors incited by A. rhizogenes are more easily regenerated into whole plants.

However, so-called "disarmed" Ti plasmids in which the oncogenic region are deleted have been constructed which seem to ameliorate the regeneration problem.

By deleting the tumor functions carried on T-DNA, utilizing NPT or other selectable markers, and culturing transformed tissues or cells in the presence of phytohormones, one is now able to accomplish transformation and regeneration of normal plants across a limited spectrum of species. Hooykaas et al (1984) recently published convincing evidence of transformation of a monocot (Narcissus cv. 'Paperwhite') suggesting that failure to produce wound galls is due to the lack of response to the tumor genes rather than integration and expression T-DNA. At present, transformation by Agrobacterium sp. is limited primarily by the ability to cultured and regenerate transformed cells or tissues. Speculating optimistically, Agrobacterium and T-DNA derived vectors will be the only tools necessary to achieve transformation in all high plants. On the down side, perhaps intrinsic limitations exist such that one or more other alternative approaches will need to be developed.

Whether needed or not, such alternatives are being developed, including direct DNA uptake into protoplasts or liposome fusions described previously. The liposome work utilized the NPT gene in T-DNA (Caboche 1984) and the direct uptake used an unspecified construction which also contained NPT (Potrykus 1985). In the latter case, an oral presentation at the UCLA/ARCO symposium on "Plant Genetics" at Keystone, CO, April 18, 1985, extremely high transformation efficiencies were reported, up to 5% of transformed protoplast-derived colonies exhibiting kanamycin resistance. The protocols were apparently effective for several unrelated species, including one monocot. However, this must be regarded as preliminary until the details of the protocol are disclosed and others have an opportunity to try to repeat the work. Given the present set of demonstrated capabilities, the outlook for achieving unconstrained introduction of new genes into a broad spectrum of crop plants would appear very bright.

The traditional view of the range of germplasm accessible to the breeder is that it is finite, and even in a state of perpetual recession due to the loss of ex situ populations through habitat destruction. Of all of the technologies discussed up to now, directed transformation holds probably the greatest long-term potential to expand the pool of germplasm in a meaningful way. In the most optimistic scenario, the breeder has in hand a set of existing breeding lines which define elite performance in a given geographical area, with the obvious goal of further improvements. In the broad sense, transformation embodies the capability to identify or otherwise generate specific genes which will deliver these improvements. Once obtained, genes are spliced to the desired on/off switch (constitutive, induced, or developmentally active promoters) and further modified for mobilization and integration into the genetic apparatus of the transformee (see Figure 1). Thus, the term "plug in" transformation has become popular to describe this process. If all works as planned, only a few simple steps are theoretically required to produce the finished product from transformed lines. Rigorous testing for at least two years and in several locations will be necessary, as it is for new candidate varieties now. Another possible application of plug in transformation in the development of finished varieties is the addition of genes which alter reproductive behavior at the end of the breeding process, for example male sterility or self-incompatibility for hybrid seed production. This is of minor significance relative to the pool of all potential genes.

In reality, we have a long way to come before transformation qualifies as a routine tool for germplasm augmentation in any significant way. Unlike previously addressed forms of technology, hurdles facing transformation include sociopolitical in addition to technical. Because this technology has no intrinsic bounds with regards to target genes, and a small subset of potential transformants might conceivably pose health and/or economic threats, the important and perplexing issue of regulation enters the picture. By now, nearly everyone is aware of environmental advocate Jeromy Rifkin's successful attempt to block efforts by University of California researchers to release an engineered bacterium into the environment for field testing. This particular bacterium was altered genetically to contain a gene from another bacterium such that it produced a substance which prevents ice enucleation at sub-freezing temperatures, a trait of obvious potential benefit. Because both the gene and bacterium already exist in nature, this particular case is arguably unthreatening. Other hypothetical examples, however, carry a perceivable degree of risk, for example, herbicide and insect resistance, along with the realm of elusive potential "side effects". The regulation issue is not a trivial one, and must be resolved as quickly as possible so that the tremendous benefits of this technology are not squandered at the expense of sabre rattling.

The regulation issue is complicated by definitions of biological entities and the domains of existing regulatory agencies. The products of traditional plant breeding have never come under Federal regulation. Recombinant DNA technology is regulated in a voluntary sense according to the Recombinant DNA Advisory Committee (RAC) of NIH. Both the FDA and EPA are in the throes of carving out niches of provenance in regulation of the release of engineered biological entities into the environment. An Ad Hoc congressional Committee formed to address this issue has taken a very strict stance on regulation. Still, no clear process on sytematically and objectively resolving this issue has emerged. One approach would be to form a non-partisan separate agency guided by a balanced committee of scientists, environmentalists, and members-at-large to regulate all matters relating to the release of genetically engineered organisms into the environment. In any event some balanced forum needs to be created soon to assist in forging the regulation machinery to assure that valuable research is not stifled needlessly.

We are challenged by a formidable array of technical problems before the full potential of transformation can be realized. In the foreseeable future, our capabilities are limited to traits of relatively simple inheritance and whose expression must be dominant, or at least semi-dominant over the existing, less desirable, condition. Since the process of transformation as we know it is consummated by the more or less random integration of sequences into the target genome, desired recessive traits will remain perpetually masked unless driven by homology to substitute directly for the dominant allele. Beyond this simple caveat lies the enormous void of genetic information on development. Most phenotypes are certainly manifest as a consequence of large numbers of interacting genes, all turned on and off in a complex orchestrated sequence. How can we adapt our simple understanding and protocols to make the vast majority of such traits amenable to transformation? While no simple solution is apparent, the needs for sustained meaningful research on the biochemistry, molecular biology, physiology, and development are again underscored. Answers to questions regarding the uptake, integration, and expression of foreign DNA and the individual steps from gene(s) to phenotype will assist in expanding the range of amenable species and in constructing transformation cassettes which will have the desired impact.

Transformation is an area plagued by poor communication. A given researcher has very limited access to outside materials and information. This problem is exacerbated by the fact that most researchers are working on their own independent genes, promoters, vector constructions, protocols, and target host organisms, although many aspects of divergent systems are common. Each step in this process is extremely time consuming and expensive, and researchers are reluctant to share even the most esoteric traits of labor without obtaining something in return, e.g. recognition or economic benefits. This applies to both public and private domains. Applications for process patents and patents on life forms or DNA sequences and plasmids hav further bound the flow of information. It is common knowledge that information is sequestered and released six months or more after confirmation to permit adequate review of protection, and to maintain a competitive edge. While this all does little in a direct and short term way to push the cutting edge faster, it must be recognized that active research in the private sector would not exist without the ability to secure proprietary protection. Through Federal granting, advisory, and regulatory agencies, researchers should be encouraged to the fullest extent possible to make non-proprietary information and materials generally available.

II. Biotechnology and Genetic Diversity

On the esoteric end of the spectrum, new biotechnological tools, particularly efficient DNA sequencing capabilities, will assist in the accurate measurement of genetic diversity and ultimately to tell us what it really means. This will, of course, make it more tenable to assess exactly how vulnerable or invulnerable we are. At the practical end of the spectrum, the fruits of research in biotechnology have typically been focused on proprietary tangibles. Plant germplasm should not be included within the realm of the items enjoying little or no accessibility. Yet everyone benefits from the availability of germplasm as raw materials for product development and as experimental materials for basic biological studies. As we have speculated in the previous section, certain biotechnological approaches hold great potential for expanding the pool of available germplasm, for example somaclonal variation, protoplast fusion, and recombinant DNA/transformation. How might these technologies be made to bear on expanding germplasm resources in addition to inevitable product focus? The potential magnitude of the issue makes this a very difficult question to grapple with. This problem can be appreciated better if partitioned into levels of complexity.

1. All existing genetic entities, including phylogenetic groups, organisms, and genotypes.
2. All potential genetic entities.
3. The large number of individual operations mandated by each biotechnological approach. It must be appreciated that many of these, at the present state of the art, are tedious, expensive, and require very high levels of training.

Each of the three general approaches has its starting point in a single, or at most two, existing genetic entities, and its endpoint in a more or less limited array of new genetic entities. In each case, a sequential pathway of a large number of steps is required. Each also has a probability of success and failure depending on the starting germplasm, the state of the materials, the skill of the researcher(s), and a multitude of mitigating factors usually summed up as "luck." Even with success, the probability of generating anything of real significance with respect to long-term genetic fitness is probably limited (although it can be argued that any new variation has unforeseeable value).

Each of these steps has a multiplicative effect on the amount of work necessary to even scratch the surface of this overwhelming task. Aside from handling input materials in bulk, there is little that can be done to bring substantial applications of biotechnology directly into the realm of germplasm enhancement.

Indirectly, however, there is much that can be done to encourage the support of germplasm efforts and contribution of proprietary materials. Acquiring, maintaining, and evaluating germplasm is a job which, like education, benefits the entire scientific community. Yet the private sector and the majority of public genetic research programs do not as a rule contribute. The challenge will be to bring these groups into the system for purposes of providing ongoing biotechnological inputs and augmenting the system in general. Perhaps, as a medium is created whereby scientists in molecular and cellular biology can come together and exchange ideas and materials with respect to germplasm, some of the general cloistering of information can be alleviated.

The following steps are highly recommended:

1. Sustained and meaningful funding of plant biochemistry, development and genetics. This will constitute a relatively small but powerful investment into the future of U.S. and world agriculture. The ability to apply the fruits of cell and molecular biology, still in their infancy, in germplasm enhancement and crop improvement to a large extent will depend on knowledge, not empiricism.

2. Encouragement of biotechnological concerns to become active constituents of the existing U.S. Plant Germplasm System. Devising some sort of "share the load" system whereby germplasm acquisition, evaluation, and maintenance can be accomplished for everyone's benefit will present a great challenge.

3. Establishment of a grant program to encourage technological inputs of all levels into the enhancement of biological diversity. This should not be limited to so called "high-tech" approaches alone.

III. Proposed Plan of Action

The main granting agencies for basic research in the U.S. include NSF, NIH, DOE and USDA/CRGO. Funding of basic plant science has been primarily through NSF and USDA/CRGO. Total funding for Plant Science research in FY 1983 was $201.6 million (Anderson 1984). Of this amount, approximately $14.5 million from the USDA/CRGO supported more or less basic plant science research. Of the entire DOE Health and Environmental research budget of $176.1 million in FY 1985, less than $200,000 was allocated for research in plant cell and molecular biology. DOE's Biological Energy Research Program invested approximately $10 million in basic plant research in FY 1984. A ballpark figure for National Science Foundation funding for Plant biology research is $52 million (Kenisey 1985). However, this is broadly defined, and the actual amount for cell and molecular biology is somewhat lower. This brings the estimated total to $76.5 million.

The U.S. National Plant Germplasm System (NPGS) is coordinated through the Agricultural Research Service of the U.S.D.A. It has represented an excellent example of shared tasks between the U.S.D.A. and State Agricultural Experiment Stations. Activities are overseen by the central Plant Germplasm Committee and the individual Technical Committees in each of the four decentralized regions. In addition, the major crops are represented by Advisory Committees made up of scientists with high expertise in those crops. They provide inputs into the system such as the accumulation and processing of information on crops, and overseeing NPGS and other domestic activities specific to the crop.

It is proposed that another committee be constituted to assist in bringing high technology and the NPGS together. Activities of the committee would include advising the Plant Germplasm Committee, interfacing the biotechnology community to the NPGS, (serving as a touchstone for the flow of information and materials between the two), devising plans to broaden the base of scientists sharing parts of the present NPGS burden, and administering a grant program to encourage technical applications in the generation of diversity, efficiency or accuity of evaluation, and efficiency of maintenance. It is speculated that a relatively small investment in this area will fuel huge returns well into the future.

IV. Congressional Mechanisms

The prominence of the U.S. in the world economy owes a great deal to investment into research and development. For example, our agriculture, the most advanced system in existence, represents a steady accumulation of accomplishment through genetic and agronomic research. Problems are appearing on the horizon which will greatly challenge our ability to sustain future generations, for example population pressures, shifts toward industrialization, depletion of fossil fuel reserves, and natural or induced environmental fluxes. Our ability to meet these challenges will depend greatly on the existence of genes, either collected in nature or created in test tubes, which condition crop performance over a broad spectrum of environments. The U.S. has established an exemplary system to dispatch the tasks of collecting, maintaining, and evaluating plant germplasm. Researchers in our Universities and private companies have made the U.S. a recognized leader in plant biotechnology both in a fundamental and applied sense. Maintaining this position and reaping potential economic returns will require a financial committment to research through our major public grant programs. The Congress can accomplish this by increasing appropriations to NSF and NIH with the stipulation that they be earmarked for worthy projects in developmental biology, genetics, biochemistry and physiology particularly as applied to plants. Appropriations for USDA and DOE for similar purposes should be increased. Lines of effective communication between granting agencies should be established or strengthened such that substantial gaps and duplications do not develop. Finally, the greatest possible tax advantages should be advocated for private companies wishing to invest their profits into biotechnological R & D. Historical perspectives have shown this to be an extremely worthy investment.

With respect to the germplasm issue, it is concluded that biotechnology in its present form will not have a great impact because it is too ineffecient and expensive. In the near term, new strategies for maintenance (see Chapter ______) is the only area of significant impact. As the biotechnological toolbox becomes continually restocked, owing to advances, processes will hopefully emerge which will permit us to engineer genetic diversity in an efficient and meaningful way.

In the interum, awareness of the NPGS, participation in policymaking, and enhanced communication among players in the U.S. biotech game can be encouraged. Further appropriations to the U.S.D.A. to establish a national Technology Advisory Committee are urged, with responsibilities listed above.

Plant germplasm provides our lifeline into the future. Our present concept of life depends on plant-based agriculture, and will continue to do so well into the future. Now is the time to recognize its importance and to move to encourage ways to preserve and enhance it, and to mold it more effectively into forms that will meet the tremendous biological challenges that stand before us.

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Contract Paper No. 5

Plant Germplasm Storage Technologies

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National Plant Seed Storage Laboratory

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PLANT GERMPLASM STORAGE TECHNOLOGIES

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ABSTRACT

The best preservation technologies can only maintain genetic diversity that has been collected. Preservation of genetic diversity requires effective application of storage technologies. Storage can maintain diversity; however, finite longevities and distribution require propagating materials. Effectiveness, therefore, depends upon both retaining viability and vigor, and in utilizing regeneration procedures without losing diversity due to using too few individuals or using samples with low viability. Application of techniques for measuring diversity are necessary before all aspects of effectiveness of preservation technologies can be assessed.

Preservation technologies can be categorized as in-vivo maintenance, in-vitro maintenance, cold storage, and cryogenic storage. Seeds and vegetative plants are the storage form of most germplasm. In-vitro cultures are supplementing in-vivo maintenance for several species. Pollen and nucleic acids may be useful for augmenting germplasm preservation in the near and distant future, respectively. Active and base collections may require different storage conditions, and strategies for improving preservation should critically assess the requirements for these different collections.

Orthodox (desiccation tolerant) seeds are stored at ca. 5°C (medium term storage: few to 20+ years) or ca. -10°C to -20°C (long germ storage: ca. 20 to 100+ years). But actual data for different species are sparse and longevities vary considerably. Storage of seed under low temperatures and low humidities prolongs viability. Cryogenic storage should give extremely long periods of viability for many materials and must be realistically evaluated, especially for base collections. Recalcitrant (desiccation sensitive) seed species are usually maintained as vegetative plants because of short seed longevity. In-vitro preservation of these species is feasible, but not yet used. Research into cryogenic storage of recalcitrant seed is necessary.

Clones are maintained if there is some desirable attribute of the line or if considerable time is required to grow a seed to a mature plant. Clonal germplasm is stored as vegetative plants, usually entirely by in-vivo maintenance. This includes orchards, some forests, and botanical gardens and arboreta. In-vitro preservation has a number of advantages and should supplement in-vivo storage, but it requires both research and development for application. Cryogenic storage of shoot-tip/buds requires research, but holds promise for base collections.

Preservation technologies will be improved and advanced by an increased understanding, through research, of all issues of germplasm. Basic problems with preservation can be improved for all types of germplasm by developing sound policies, more fully utilizing existing tehcnologies, developing rigorous training programs, and more critically assessing performance. Improvement requires strong guidelines, action and additional support.

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PLANT GERMPLASM STORAGE TECHNOLOGIES

I. Introduction

Plant germplasm comprises the total array of living plant species, sub-species, genetically defined stocks, genetic variants and mutants whose continuing availability is important for societies present and future health and welfare (77). The extent of materials considered germplasm for a given crop depends upon the crop. Thus, potato germplasm, because many of the tuber-bearing Solanum species intercross, consists of some 160 or more species and all lines contained within. Maize germplasm consists of only a few species, but comprises many adapted land races.

The concept of biological diversity includes both genetic diversity, the amount of genetic variability among individuals in a single species or genus, and species diversity, the number of species in a particular community (77). Since major concern is with crops for food, fiber, fuel, shelter, and medicinal purposes, the concept of genetic diversity within a species is more pertinent. Species diversity is an ecological concept which is important not only because these species may represent future important crops, or contain information eventually useful for crop improvement, but because communities provide benefits relating to sustainable land development and environmental stabilization (e.g., erosion control, recreation, watersheds, wildlife habitats).

Advances in knowledge of gene structure, function, and manipulation as well as increased understanding of incompatability barriers and ways to overcome them herald a new era for use of genetic manipulation for crop improvement using genes from taxonomically distant species (40,46,84). Thus, we should adopt the broadest definition of germplasm of a given crop for collection and preservation purposes. The question of delimitation is not trivial for there are great concerns in effectively utilizing limited funds for the collection, maintenance, evaluation, and distribution of germplasm accessions. Questions of what needs to be collected, how much, from where, and where maintained are very difficult to answer even on a crop-by-crop, or species-by-species basis.

Ecologists, geneticists, and plant collectors of many crops have repeatedly warned about the rapid disappearance of land races, primitive cultivars, and wild species (36,37). Clearly the need is to adequately collect materials now, especially in endangered areas. The United States is poor in crop relatives and land races of most major food crops, and thus, collections must be made in many other countries (48,66). These foreign expeditions require extensive planning and international cooperation for success. Fortunately, concern about maintenance of genetic diversity is worldwide and both the National Plant Germplasm System (NPGS), and the International Board for Plant Genetic Resources (IBPGR) have laid the groundwork by sponsoring numerous meetings, symposia, training sessions and publications on important issues of germplasm conservation for many crops (63,79). Major food crop diversity can be sampled, but the adequacy of these collections remains a question (37,39,109). Collections for emerging crops and plants producing medicinally important compounds are much more difficult to sample because little is known about the genetic composition of these plants and their populations. Land races and cultivars do not exist--rather heterogeneous wild populations are typical, but are usually inadequately sampled (57). Problems with sampling rare wild species are even greater.

The genetic diversity of a species consists of geographical diversity, interpopulation diversity, and intrapopulation diversity (50). Although there are strategies for sampling to obtain individuals representing as much of this diversity as possible (2), practical restraints limit the amount of diversity collected (57). It is imperative that the diversity that is collected be maintained through the best preservation technologies available. Plant propagules have a limited longevity under storage conditions. Limited longevities and distribution of materials to the user community necessitate periodic replenishment of the stock. Sound reproduction strategies using adequate numbers of parents must be utilized to insure no loss of genetic diversity during this phase. Thus, preservation embodies both storage and regeneration.

Although genetic diversity is an understandable concept, measures of diversity are not easy to define. Diversity is evident by morphological, phenological, biochemical, and physiological differences, but the measurement of it is limited by the tests we apply. As an example, disease resistance to an, as yet, unrealized pest, cannot be evaluated and the diversity for resistance remains unrecognized. This signifies the importance of adequate evaluation to gain measures of diversity. When better measures of diversity exist, better storage technologies and regeneration strategies can be designed and implemented. It is difficult to separate the importance of storage for retaining diversity from other aspects of maintenance, especially the regeneration phase. Inadequate storage and improper regeneration definitely lead to the loss of genetic diversity (89,91,96,97). With good preservation technologies, the objective is to maintain the genetic diversity that was initially in the collected accessions, but obstacles are abundant. Diseased materials, materials grown and multiplied under adverse environmental conditions, and genetic traits linked to unfit genotypes may yield propagules which do not store well under the conventional system, and if grown out in a regeneration cycle may not contribute to the next generation (for example by not flowering, especially in an inclement environment). The use of alternative storage technologies may retain viability in these variant materials in many cases; however, these technologies must be creatively designed and used.

The maintenance of species diversity is more difficult. One must not only maintain the species diversity in a community but also the genetic diversity of each component species. Species diversity could be maintained with existing storage technologies if adequate samples of genetic diversity within each species of the community were collected. Since most collections probably represent inadequate sampling, species diversity at best is a biased sample of the native, wild community. The lack of adequate measures of diversity raises the question whether current collections are preserving acceptable levels of genetic and species diversity.

II. Germplasm Preservation--Technology Assessment

The need for germplasm preservation of diverse species has been amply stated (36,37,114) and efforts have and are being expended nationally and internationally to collect germplasm from centers of crop diversity and endangered growing areas (54,72). These collections of land races, primitive cultivars, and wild species must be maintained, multiplied and evaluated to assure their avabilability for future plant improvement.

What storage technologies are available and used, and what alternative technologies exist that may allow for more efficient preservation? The concept of an active collection for a crop as contrasted to a base collection needs to be emphasized first because different storage technologies may be applied to each. This concept usually relates to lines maintained as seed. An active collection consists of materials available for distribution, usually for application to breeding programs (26,80). The term working collection is sometimes used as a synonym for an active collection but here we restrict this term to materials held and used by breeders. The distribution and use of propagules along with decreases in viability during storage deplete stocks and replenishment is necessary by prescribed regeneration procedures that differ from species-to-species.

A base collection is essentially a duplicate of the active collection, but it is held in long-term storage as a back up should there be a loss of the active collection (26,80). These stocks are generally not distributed but do suffer viability decline during storage. Obviously, storage strategies that allow the greatest longevity are desired for base collections. Loss of genetic diversity and increased incidence of mutation are associated with loss of viability (90).

Storage longevities required for active collections somewhat depend upon distribution requests, and thus, materials may not require conditions that give the longest storage. In practice, requests for different accessions vary considerably. Within an active collection, the seed which is grown out for crossing to regenerate the seed stocks should have the best storage possible since it is used to perpetuate the genetic diversity for the user community.

Thus, different storage technologies for active and base collections are highly desirable. Two examples are illustrative. Cryogenic storage of seed may give extremely long lifetimes useful for a base collection (90,105,106); however, space considerations for the greater numbers of seed required for an active collection may dictate that 5°C storage in vaults is sufficient for distribution seed, and -20°C storage sufficient for seed used to regenerate the seed lot. For woody clonal materials, orchards (field genebanks) or in-vitro plant cultures are preferred for the active collection because budwood cuttings, or cultures can be supplied with little delay. A field genebank can never form a satisfactory base collection (60). A base collection of cryogenically-stored shoot-tips or buds would be space and energy saving, but would not be in a form which can be directly sent to a user.

For purposes of this paper we consider that germplasm preservation consists of the storage technology per se and the regeneration (multiplication) phase. Both are the responsibility of the genebank and are intimately connected.

A. Germplasm Acquisition and Maintenance

In the United States germplasm acquisition began on a rather informal basis when the first colonists arrived bringing with them seeds of various crops. After the establishment of the new republic, ambassadors or consuls in foreign countries were encouraged to send seeds of new plants which might be of use in American agriculture. In 1839 the first grant of $1,000 was made for plant introduction and in 1897 the Office of Foreign Seed and Plant Introduction was established. A sequential numbering system for documenting germplasm accessions was begun in 1898 and thus the beginnings of the National Plant Germplasm System were laid down (103).

1. Reasons for storage

Although nearly half a million Plant Inventory (PI) numbers have been assigned since 1898, a number of these accessions, especially the earlier acquisitions, have been lost. James (63) estimated that 70% of the soybeans, 98% of the clovers, and 66% of the oats introduced were no longer available. This may be an overestimate because holdings by breeders were not considered. Loss occurred because the complexity of germplasm issues were not generally appreciated and appraised. No long-term storage facility existed. Breeders who retired or died often had no one to take over their materials, or germplasm stocks were evaluated for the specific traits and then discarded. This is still a problem. The thinking then was that we could always go back to a location and collect more materials. With the second World War and the political aftermath, collecting expeditions could no longer be carried out at will in some locations (for example, China) and thus the idea of a long-term seed storage facility was conceived.

The need for preservation of germplasm is greater than ever. Germplasm is being lost world-wide at an alarming rate due to such factors as: (a) environmental changes (Sahel desert, clearing of trees in the Amazon rain forest; (b) natural disasters (flood, fire, earthquakes, volcanos); (c) loss of agricultural land to urbanization; (d) green revolution (replacement of land races with new cultivars and hybrids--monoculture); (e) lack of recognition of wild relatives of domestic species resulting in their extinction through carelessness, or elimination because they may be disease carriers. Political problems in and among countries may restrict access and exchange of germplasm.

The enormous task is to acquire this endangered germplasm, before it is lost. The best we can hope for is to acquire the material and preserve it until such time that evaluation can be accomplished. Long-term, ex situ storage in base collections offers the best chance of maintaining the genetic variability still available.

2. Criteria for acquisition

All of the germplasm of all species cannot be preserved. Thus some criteria must be established for acquisition. Germplasm for a given crop plant consists of five kinds: (a) cultivars in current use; (b) obsolete cultivars; (c) special genetic stocks of various crops such as resistance stocks, genetic and cytogenetic material, induced mutations, etc.; (d) primitive varieties or land races; and (e) wild and weed species related to cultivated species (35).

Seed of self-pollinating and open-pollinating cultivars in current use are routinely acquired and preserved while hybrid cultivars are not [policy statement, U.S. National Seed Storage Laboratory (NSSL)]. Obsolete cultivars may or may not be in existing germplasm collections. If they are not in the collection now, there is limited prospect of finding them. Special genetic stocks such as those for tomato, peas, corn, and barley are presently maintained by individual curators with financial support from the USDA and others. These stocks are generally well-maintained and are used extensively by plant geneticists for gene mapping and for various genetic and physiological studies. As new genes are discovered they are added to the genetic stock collections. This leaves the last two categories (d and e) for additional collection and widening of the genetic base for a given species.

In the United States germplasm collection missions are developed by individual scientists, usually curators or plant breeders, and funding is available from the USDA after review and approval. Generally 4 to 6 plant exploration missions are funded annually from a budget of approximately $50,000 per year provided by ARS. The criterion for acquisition is largely the perception of an individual or a group responding to the needs of a particular crop or species. All germplasm collected on USDA sponsored missions becomes part of the NPGS and is freely available to plant scientists everywhere. Private companies, individuals, and other institutions (for example, botanical gardens) may sponsor explorations for their own benefit.

The IBPGR has also sponsored numerous expeditions over the past 10 years (59,72). The outcome of these collections (over 300 missions in 88 countries) has resulted in over 100,000 samples being deposited in genebanks. Future efforts by the IBPGR will emphasize individual crop genepools and specific collections of wild, related species. Also the intent is to "carry out more field surveying so that much more specific collecting can follow as a result of eco-geographical data analysis." As collecting work slows greater emphasis on characterization of the materials should result in better utilization.

Another important mechanism for acquiring germplasm involves exchange of materials with other germplasm facilities, governments, and individual breeders. Through the efforts of the NPGS and the IBPGR such exchanges have been both encouraged and facilitated through various funding mechanisms.

3. Evaluation

A serious deficiency in our overall germplasm effort has been germplasm evaluation (18,86). Underutilization of accessions is mainly a result of lack of sufficient data on existing material. The time and effort required by plant breeders to screen all of the samples for a given species for a specific trait is prohibitive, especially when the pressure to produce a new and better cultivar is so strong and academic and financial rewards are based on such production. The situation has been improving. International research centers, such as CIAT, CIMMYT, IRRI, etc. have screened many accessions for up to 30 or more plant characteristics important in agriculture, chiefly disease and pest resistance, yield, and quality factors (21,23). There is no easy way to assess thoroughness and accuracy in evaluation of a given collection. The users experience with materials and supplied data will determine reliability.

In the U.S. the four regional plant introduction stations have always included preliminary characterization as part of their maintenance duties for working collections of seed. This, however, is not sufficient evaluation and more extensive efforts are necessary. Recently two developments have sharpened the focus on these efforts. The first is the development of the Germplasm Resources Information Network (GRIN) which provides a readily available computerized database for handling plant evaluation data (66). The second development is the formation of Crop Advisory Committees (CACS) (now 34 in number) one function of which is to identify and prioritize characteristics of importance for evaluating germplasm accessions. Evaluation is done mainly by crop breeders. Financial support has been increased recently and prospects for further increase appears encouraging.

Industry has long recognized the need for more evaluation. Recently Pioneer Hi-Bred International provided a $1.5 million grant over 5 years to the USDA-ARS for evaluation of Latin American maize germplasm (6). Additional support of this nature could result in an accelerated pace for germplasm evaluation and subsequently its more widespread utilization.

Characterization of germplasm is necessary to determine and test optimum regeneration cycles to insure that diversity is not lost in storage or regeneration phases. Characterization may consist of electrophoretic analyses of protein or restriction DNA fragments, possibly combined with morphological measurements. Nonspecific protein and isozyme characterizations have been used in many crop species, perhaps best so for maize germplasm (43,81). Considerable methodology exists but application to diverse materials other than major crops is sparse. However done, the characterization allows asking questions related to changes in populations, particularly heterogeneous ones, during storage or regeneration. Extensive characterization may not be necessary for all accessions of a species but merely for a sufficient number to ascertain the optimal preservation conditions. If money, manpower, and techniques were sufficient, it would be desirable to assure that the diversity in each accession were maintained.

4. Duplication within a collection

The major problem with duplication of germplasm accessions resides in the inability to identify absolute duplicates, and hence costs with storage and regeneration are increased. When evaluations are done differences among accessions sometimes result in identifying unique accessions; however, when can you ever say that two accessions are exact duplicates? Some additional test may prove two otherwise identical samples are, in fact, different.

Duplication within a collection occurs in many ways. Mislabeling or human error during seed increase can result in duplication of some samples (and possibly loss of others). Different names for the same cultivar or repetitive sampling of the same material (germplasm collection mission) can result in duplication. In some cases germplasm samples have been distributed to users or other genebanks, have then been redistributed, perhaps renamed or new identification numbers assigned, and then returned to the original source as new accessions.

In the above discussion we have been thinking in terms of exact duplicates. However, it may be that very minor differences between accessions, for example, differences in gene frequencies or maturity differences of 1 or 2 days, may not be of concern. In these instances duplication would be considered to be present. Other times such differences are very important and, thus, duplication is not present. Attempts to identify duplicates have focused on measuring numerous plant characteristics under field grow-out or laboratory conditions. Computer clustering analysis can then calculate "genetic distance" among all accessions (87). Also techniques such as electrophoresis of proteins and enzymes have been used to "identify cultivars" (17,81). Again the results of these analyses may tell us when two samples are different, not if they are identical. However, for the purpose of determining genetic variability (diversity) within a collection, they are helpful tools.

Some duplication within a collection, especially at the gene level, is highly desirable. In the case where an accession is lost, the genes at least would be saved, albeit in different combinations. Duplication among collections is also necessary in the event an entire collection is lost through some diasaster. Within the NPGS the policy is to duplicate the active collection in the base collection at the NSSL. However, at present numerous samples are present in only one or the other collection.

B. Types of Preservation Technologies Available

The preservation technologies available can be termed *in-vivo* maintenance, *in-vitro* maintenance, cold temperature storage, and cryogenic storage. Germplasm from a given species may be stored by any of these technologies; however, the nature of the crop and the propagule, the genetic breeding system, and ease of storage currently favor one or two technologies. Preservation technologies are often classified as *in situ*, where conservation occurs in the natural state and *ex situ* where conservation occurs in some manner other than the natural community (50,83).

1. In-vivo maintenance

Preservation of germplasm in this manner involves preservation of the vegetative plant and has several forms. One form is the ecological or biosphere preserve best envisioned for wild species where maintenance is under natural conditions (*in situ*). Other forms involve plantations (*ex situ*). The botanical garden utilizes both sheltered structures and open areas and entails considerable management of species composition and environment. Other controlled plantings include orchards, some forests, perennial gardens of herbs and flowers, and root and tuber plantings.

2. In-vitro maintenance in the growing state

The concept of *in-vitro* storage is still evolving but for purposes here is considered to be the growing of entire plants (plantlets) in glass or plastic vessels under sterile conditions. With proper methods lines are isolated in a disease-free (tested) state and maintained as such. Storage conditions are under light at normal growth or reduced temperatures and may involve growth media modifications. *In-vitro* plants are initiated from either meristems or buds and are propagated through divisions; thus, lines are maintained in clonal fashion.

3. Cold temperature storage

For discussion purposes, this is defined as storage at temperatures ranging from about +5°C to -20°C, the range possible with single stage compressor refrigeration systems. Seed is the propagule usually stored at cold temperatures, although some cuttings, tubers, roots, etc. from clonal lines can also be maintained for periods of months at temperature of 0 to 10°C. The increased use of in-vitro cultures has stimulated studies on their storage at reduced temperatures and under minimum growth conditions (56,60).

4. Cryogenic storage

Cryogenic storage refers to storage of propagules under very low temperatures. Ideally, temperatures less than about -130°C are desired because of absence of any liquid water, very low molecular kinetic energies and extremely slow diffusion (74). Chemical reactions (e.g., metabolism), thus, occur very slowly and storage longevities are postulated to be extremely long--limited only by the buildup of genetic lesions due to background irradiation (10). Storage in liquid nitrogen (LN, -196°C) or in the vapor above LN (ca. -150°C) is usually envisioned because it is a relatively cheap cryogen. The storage of seeds, pollen, and small shoot-tips and buds in LN is projected to be useful for preservation of base collections once research and development problems are overcome (105,112,115).

C. Propagules for Germplasm Preservation

The following are the general categories of propagule used for germplasm storage. For a given species one or more types are used to store diversity. The particular characteristics of the species or crop determine which form is stored.

1. Seeds

Seeds are the most common storage propagule for germplasm preservation, although they differ among species in longevity and ease of storage. Long periods of storage are desired to reduce problems associated with seed regeneration schemes, including cost of regeneration (cost, time, space) and risks of loss due to disease and environmental problems (89). Short-term seed storage is usually under ambient conditions, medium-term storage at ca. 4°C and long-term storage at -10°C to -20°C. For example, longevities for rice seed under short-, medium-, and long-term storage are about 5, 30, and greater than 70 years, respectively (21).

Seeds are classified into orthodox seeds (those capable of retaining viability after being dried to less than about 5% moisture content--also termed desiccation tolerant seed) and recalcitrant seeds (those losing viability after being dried below a critical limit, usually about 12-30% moisture--desiccation intolerant seed) (22). Desiccation-intolerant seeds are also termed short-lived seeds. Most temperate crop species have orthodox seeds. Orthodox seeds are usually dried to moisture contents of 5-7% and are then sealed in moisture proof foil laminated bags for storage at between about 5°C and -20°C (15,26,67). Care must be taken to be sure seals are complete and no punctures occur. Other storage containers may suffice (26,62). Accurate seed viability tests and methods of breaking dormancy are necessary for evaluating longevity (30,67). An additional problem with seed preservation of wild species, especially those not common, is the lack of research to establish viability testing procedures.

Many studies have shown the importance of seed moisture content and storage temperature for retaining viability in orthodox seeds (14,90). Lowering the seed moisture content to 5-7% and lowering the storage temperature increases longevity for many species, but data are limited for different species, especially with storage at subzero temperatures. Approximations based on a number of assumptions suggest that seed from many species will have longevities of about 100 years when stored at -20°C (90,91). Longevity can also be affected by genotype and by environmental factors during ripening, harvesting, and drying of the seed before storage (14,91,104). Care in handling seeds is necessary during the initial collection period (104) and subsequent regeneration cycles.

Orthodox seeds at low moisture levels often retain the same percent germination after LN treatment as before; hence, extended storage for more than a century may be possible (105). Some limitations on LN use for orthodox seed may exist and may relate to the seed coat, seed oil content, and seed moisture content. One group of orthodox seeds are desiccation tolerant, but sensitive to low temperatures (105). Many temperate fruit and nut seeds (Corylus sp., Juglans sp., Prunus sp.) exhibit this characteristic, although the extent of desiccation tolerance, the limits of low temperature sensitivity, and mechanisms of injury need further study.

Recalcitrant seed are produced by aquatic species, large seeded species, species native to tropical areas, and some temperate zone tree species (e.g., oaks) (22,69). Coconut, cocoa, mango, nutmeg, and rubber plants are examples of economic species with recalcitrant seed. Adequate tests for true recalcitrance should be established and used (93). For example, citrus species, formerly thought to be recalcitrant, were shown to be dormant instead (90). The storage of species with recalcitrant seed poses considerable problems in retaining genetic diversity. To retain viability, recalcitrant seed are stored at as low a temperature as possible under conditions which retain the relatively high seed moisture levels and assure a supply of oxygen for respiration. Some recalcitrant seeds, however, are chill sensitive. The few weeks to few months storage obtained with these conditions are too short for adequate germplasm preservation (92). Thus these species are usually stored as vegetative plants, but if diversity is considerable, many individuals must be conserved, resulting in large space and maintenance costs.

Axillary bud cryopreservation from in-vivo vegetative plants and/or in-vitro plants might be useful for base collection storage of recalcitrant seed species. But again, if the particular species is highly heterozygous and diverse, many different individuals are necessary to preserve diversity.

The inability to tolerate desiccation and the chill sensitivity of some recalcitrant seeds necessitate research into the mechanisms of injury and methods of alleviating it before long-term storage will be possible. Cryogenic treatments involving cryoprotectants may provide suitable procedures in the future, and is a suggested approach, possibly for excised embryos (44,69). However, no procedure so far has been successful for the reproducible cryogenic preservation of recalcitrant seed.

2. Pollen

Pollen is not a conventional vehicle for germplasm preservation, but in many species there is considerable interest and information on pollen preservation for breeding purposes (112). At present pollen storage is mainly for convenience in crossing materials that flower at different times. A population of pollen grains collected from genetically different individuals would contain the nuclear genes within that population; cytoplasmic genetic factors, however, would not be present.

Pollen can also be grouped into types that are desiccation tolerant and desiccation sensitive (112). The tolerant types store best when dried to a low moisture content and are kept at low temperatures, analogous to orthodox seed. Desiccation tolerant pollen survives cryogenic exposures. Desiccation sensitive pollen (for instance, pollen of many members of the Graminae) loses viability during drying and cannot tolerate temperatures where its cell water freezes. Cryobiological strategies have given some success with desiccation sensitive pollen, notably maize pollen (12), but once again data are sparse.

Storage of pollen conserves space compared to seed or clonal storage, and could be useful as a supplement for a base collection of vegetatively maintained lines, for example, species with recalcitrant seed. Species with recalcitrant seed do not necessarily have desiccation sensitive pollen. Pollen alone, however, could not form a satisfactory base collection (60). Considerable information on stability and longevity at subzero temperatures is also needed before practical storage could be used.

One limitation to the use of pollen as a sole propagule for base storage is the difficulty in regenerating whole plants directly from the pollen. Regeneration from free pollen culture is being studied in several species for production of doubled haploid plants (11). The future may bring bring methods of application to pollen from all species. Problems of diversity loss using pollen storage will still occur. For example, in regenerants traits linked to a lethal survive in the heterozygous state but are lost in individuals homozygous for the lethal trait.

3. Vegetative materials

The other major storage form of germplasm besides seeds is the vegetative plant itself. These plants are maintained *in-vivo* in greenhouses or outside plantings. Various plant parts such as cuttings, budwood, root divisions, and tubers are used for distribution purposes. Maintenance of vegetative plants of woody materials requires considerable space and cost, and loss due to disease could be expected to occur unless extremely vigorous management practices are utilized. Herbaceous clonal materials, such as potato, can be maintained within greenhouses or screenhouses where phytosanitary conditions are more stringent.

There are often tremendous adaptive characteristics within a species, associated with survival at different latitudes, longitudes, and altitudes. Growth of these materials as vegetative plants at single locations may place certain individuals under environmental stresses and decrease fecundity, and may present problems in evaluation and characterization of germplasm.

Survival of _in-vivo_ axillary buds, shoot-tips, and stem sections after cryogenic exposure has been reported (68,98), and may be useful for base collections of species which cold acclimate, especially woody materials. Research is needed to answer many questions concerning genotype stability, grafting potential, rooting potential, and physiology of the parent plant.

4. In-vitro plant culture

Plant microculture (defined here as plantlets maintained in _in vitro_) has been advocated for preservation of a wide array of species, usually those for which clonal maintenance is either necessary or desired (28,56,60). Indeed, with the current rate of progress in plant microculture technology, virtually any species will grow _in vitro_ (4,24,32,101), but indiscriminate application is neither practical nor desirable for germplasm preservation. Care must be taken to apply this technology to species which absolutely require clonal maintenance. Reasons cited include maintenance of heterozygosity and adapted complexes, inability to easily set seed, and long juvenile stages (especially in woody lines) (28). Certainly for some species seed can be used as the base collection while clonal materials can form the working collection (e.g., apple) (60).

Storage of plant microcultures for germplasm preservation occurs under either normal growth conditions, reduced temperatures, or on a growth-restrictive nutrient medium at ambient or refrigerator temperatures (56,115,116). Storage in this manner usually gives longevities of weeks to months before subculture is needed.

The long-term storage of clonal lines could be accomplished cryogenically with isolated shoot-tips and buds. There is evidence that shoot tips and buds can survive LN exposure if plants in the appropriate stages of growth are utilized and if a strict protocol is followed, including proper pretreatment, addition of a cryoprotectant, avoiding supercooling, using optimum cooling/warming rates, post-thaw recovery, and development of suitable growth media which allow plant regeneration from the bud (68,98). Research has demonstrated that shoot-tips from herbaceous lines (potato, carnation, pea, cassava, and strawberry) and from woody lines (apple) can survive LN treatment. Many questions remain, among them whether a single procedure works well for a diverse array of genotypes, and if plant regenerants are completely normal after treatment.

Based on present knowledge, after LN treatment shoot tips from herbaceous lines either form callus before shoots are regenerated (68,111) or form adventitious shoots at different locations within the tips (49,68). Both increase the chance for somaclonal variation (99,100). LN-treated buds from hardy or acclimated woody lines may develop directly into a plant, but whether this occurs for all species cannot be stated because of so few studies.

As an alternative to ice formation during low temperature exposure, vitrification (glass formation of system without actual ice crystallization during the exposure to low temperatures) is being studied for retention of viability in animal systems (33). This possibly could eliminate certain injurying events during cooling/thawing and increase the allowable size of the propagule being treated. High concentrations of cryoprotectants are necessary and must be examined for general toxicity. This is an intriguing possibility for cryogenic storage of plants that is yet unexplored.

The use of the term in vitro may also refer to cells, tissues, or organs in culture, but their use for germplasm storage is not feasible at the present time due to cytological instabilities in culture systems and the virtual ubiquity of somaclonal variation in regenerated plants (60,99,115,116). Embryogenic systems may be relatively stable compared with adventitious, shoot-regenerating systems and, therefore, more attractive for genetic conservation. Once reproducible regeneration is possible from a range of genotypes and somaclonal variation is restricted (or controlled), the potential for using cells or protoplasts of some species for storage (especially cryogenic storage) may be feasible.

5. Nucleic acid preservation

In the past 10-15 years there have been tremendous strides in manipulating genetic material. Genes can be located, excised, re-inserted, and in some cases apparently retain somewhat normal function. Gene exchange by molecular techniques among diverse biotypes is being explored. Nonetheless, a better understanding of gene structure, function, and regulation, along with interactions and interdependence on other genes, is needed for any consideration of application to germplasm preservation, but the future appears bright (85). Germplasm preservation technologies for the future may well include the preservation of the isolated genetic information (DNA and/or RNA) from the cell. In terms of present understanding, the preservation (of the DNA) could be as a lyophilized extract or a frozen solution at very low temperatures. Alternatively, a compete array of restriction fragments of the DNA could be introduced into other organisms (bacteria, yeast) and these stored at very low temperatures. Many microbes are easily cryopreserved and even successfully freeze-dried (9).

With our current understanding many problems must be overcome before this technology is utilized in any form for germplasm preservation. Two examples are the requisite interactions between organellar genes and nuclear genes and the complexity and size of certain functions and controls to be transferred. It is doubtful that nucleic acid preservation would ever be the sole means of germplasm preservation, but it could form a useful adjunct to existing technologies. Careful thought is essential in defining its use for future strategies of germplasm preservation. At present DNA technologies hold more promise for characterization and disease indexing of germplasm (85).

D. Diversity of Plant Germplasm

Any consideration of germplasm storage must deal with tremendous numbers of different plant species. Currently, crop species grown for food or fiber purposes occupy most efforts, and indeed, the need for increased productivity because of enlarging populations and for quality of products because of evolving pests and disease makes such a priority justifiable. Increased studies of genetic maintenance of forest species, likewise, is strongly argued because of their economic value. But germplasm preservation should also deal with wild materials, not only as they relate to domesticated plants, but also because of potential use as foods, medicinals, or ornamentals.

1. Genetic systems

The maintenance of genetic diversity depends upon reliable regeneration as stocks are depleted due to distribution and declining viability and requires knowledge of many aspects of plant genetics. Population structure determines sampling strategies for both domesticated and wild species (2,82).

For example, many cultivated grains are inbreds (self-pollinated) and are usually highly homozygous, although the populations may be quite heterogeneous. As a contrasting example, many fruit and vegetable species result from outcrossing and have a number of incompatability barriers to facilitate this; these populations are heterozygous and often heterogeneous. Collection and regeneration schemes must utilize such knowledge for germplasm preservation. Each species needs to be assessed to determine the best procedure for maintaining maximum diversity.

The genetics of most crop plants is well known, but manipulation of diverse germplasm by emerging classical and genetic engineering techniques is in its infancy. The knowledge of the genetics of wild species itself needs study before preservation and evaluation of genetic diversity are effective. Knowledge of the population structure of an accession is important for assessing factors that might alter the genetic composition (19,95). For example, several factors affect the maintenance of composition of a heterogeneous accession (Fig. 1) and must be considered in regeneration and preservation schemes. A homogeneous accession, however, is uniform and regeneration schemes would not have problems in differential survival, productivity, and maturity. Sample size, therefore, could be smaller. Recommendations for number of seeds for an accession in long term storage are at least 3000 to 4000 for homogeneous accession and 4000 to 12000 for heterogeneous accessions (26,61).

2. Plant diversity

Categories of crops often used (Table 1) are artificial groupings based upon economic usage and, as such, the members may not have the same breeding systems and are not necessarily stored by the same technology. Detailed discussions of the genetic resources for specific crops (mainly seed crops) can be found in a series of directories published by the IBPGR and in annual reports from many of the genebanks. The IBPGR has coordinated a world-wide network of base collections, now some 30 collections for 34 crops, and is actively devising a similar network for active collections. A current list of these genebanks is available from the IBPGR (47).

Active collections are existent worldwide for most major crops (86) and in each group other than cereals and grain legumes both seeds and vegetative plants are maintained for some component species. Base collection strategies and concepts are not well developed for species where clones are the predominant economic form or where species with recalcitrant seed exist. The storage technologies used for each group depend upon species but in general cold temperature storage is used for the orthodox seed of most groups with cryogenic seed storage in either the research or development phase. In vitro storage is being routinely used for some lines in some groups, but research and development phases are more prevalent. Appendix A provides information on preservation for the major food crop groups. We should emphasize here that there is no way to assess performance of the many genebanks worldwide with regard to reliability in either maintaining storage conditions or in preserving the diversity collected.

E. Evaluation of Technologies

The previous discussions of storage technologies, propagules, and the plant kingdom diversity necessarily included concepts of evaluation. Points to be addressed now are effectiveness in terms of cost, viability and maintenance of genetic stability, and problems of application.

1. In-vivo maintenance

Evaluation of the overall effectiveness of in-vivo maintenance is difficult; however, certain conclusions appear obvious. Ecological preserves, while probably effective for some wild species, appear to be too demanding to apply on a broad scale, given the many species and many communities existing in the world, the variation occurring within a species range, and the unique environments required by these ecotypes (38). Vast numbers of preserves would be required in many different countries and would be extremely costly to organize and operate in view of the close inter-governmental cooperation needed and the current rate of destruction of many habitats. Distinct strategies and decisions must be made on what will be preserved. Management even in ecological preserves must occur, consisting minimally of adequate security measures and observation of materials. Although evolution could continue in ecological preserves (assuming population size and gene frequencies are adequate) (38), possibilities of loss through disease and natural disasters are not eliminated. No country could preserve all species maintained within its borders and, thus, political problems of sharing germplasm could arise.

Forest resources currently are maintained similar to ecological preserves, but are usually managed to a considerable extent. Again sites are prime, often needed for other purposes, and hence are rapidly being lost. Many valuable tree species have been lost due to excessive harvesting (83).

The botanical garden concept for herbaceous and woody species could preserve selected species in vegetative form, but, even here, the numbers that could be stored are few relative to the numbers required to maintain species diversity. Organization among botanical gardens could allow division of labor such that more total species could be stored and overlap minimized. The Center for Plant Conservation, headquartered at Arnold Arboretum, Harvard University, is an effort in this direction (34). Cold or cryogenic storage of seed obtained from collecting expeditions might be the most feasible way for germplasm preservation even at a botanical garden. Arboreta face similar problems in their attempts at preserving tree and shrub germplasm. Both botanical gardens and arboreta have high maintenance costs, problems with disease and pests and with growing materials outside of their environment, and priorities in materials displayed to their constituents (77). Botanical gardens and arboreta usually do not distribute large amounts of materials.

Orchards and other clonal stores are still more managed than forests and are commonly used, especially if the numbers of individuals are small. Again, disease and pest damage are real threats and not all genotypes thrive in a given environment. Thus, multiple sites are needed along with plantings of several individuals of a given clone. Better inter- and intra-governmental management is needed to avoid loss. Many *in-vivo* collections especially of fruit and nut, and ornamental species, have been maintained by individuals at federal, university, or industrial facilities and upon retirement, death or project change these materials were lost. For example, the recent closing of a plant introduction station resulted in loss of several clonal germplasm accessions (42). Similar losses also occur for seed materials. Increased awareness of these potential germplasm sources and plans for germplasm retrieval and inclusion in base storage must occur before loss, without regard to current economic value.

2. *In-vitro maintenance*

Experience with this relatively new technology is limited; thus, time and cost analyses are lacking. An international database on *in-vitro* conservation has been established to improve information flow among workers with common interests (113). Combinations of *in-vivo* and *in-vitro* maintenance are being used in the U.S. Clonal Germplasm Repositories for fruit and nut crops and should produce cost effectiveness and acceptability information.

Development and implementation is restricted by inadequate information on optimal storage conditions, on application to diverse genotypes and species, and on genotype stability. The advantages of microculture are increased safeguards in maintaining a disease-free state, more rapid multiplication rates, ease of rooting (especially for woody materials), less maintenance requirements, possibly less labor input, and ease of germplasm exchange and shipment both within and among countries (56,115). Potential problems include establishment procedures, which are often cultivar specific; explants from mature woody plants are difficult to establish in culture in contrast to that from juvenile plants; dormant periods are sometimes required; diverse genotypes may not survive in the same physical and chemical environment; uncertainty of occurrence of somaclonal variation, even from tip culture; and storage of all materials in one location (28,56,115).

Until acceptability is gained, usually through experience over time, in-vitro storage is now best utilized as an adjunct to in-vivo storage. The technique has not been used enough to provide answers to questions of extent of duplicate samples, duplicate locations, and handling needs. Laboratory, growth, and transfer facilities are moderately expensive and must function as specified. Adequate identifiers of each line (e.g., electrophoretic profiles) must be established and lines periodically examined to avoid identification errors.

Many developed and developing countries are exploring in-vitro storage technology for clonal lines (e.g., potatoes, strawberries), and have relied upon the international centers and IBPGR for guidance and for information exchange to more directly apply in-vitro culture to genetic conservation (60,99). Sharing of sound technology to preserve germplasm does not appear to be constrained by social, institutional, or political barriers. Sharing of germplasm itself amongst countries may be potentially political, and exchange of in-vitro materials could be affected.

3. Cold temperature storage

Cold storage is used throughout the world for germplasm preservation of seed. Storage units must be adequately designed to operate continuously and efficiently at the required temperatures. Modern mechanical refrigeration systems are reliable and recommendations exist for protocols, cold room design, and emergency procedures (26). Modifying older units may present problems. Experience at the NSSL has shown that maintaining -20°C with mechanical chambers originally designed to operate at 4°C is often difficult and not always reliable. Although large walk-in chambers are usually desired, deep freeze chests are adequate for small collections (29). Refrigeration in tropical areas poses problems because of high temperatures and humidities.

The major consideration is what temperature should be used. Current data on seed longevities at low temperatures are insufficient and innovative research is needed to determine longevity/temperature relationships for different germplasm. Lowering of the storage temperature to approximately -20°C should greatly increase longevity, thus making costly regeneration cycles less frequent.

Although between 3000-12000 seeds are recommended for long term storage of an accession, many accessions in base collections are considerably smaller, often inadequate to maintain diversity. Seed handling, packaging, germination testing, and shipping have been evaluated and somewhat standardized. Seed germination testing, itself, is costly in terms of manpower and in sacrificing valuable seeds for the viability estimate. Viability testing could be less frequent if storage is at lower temperatures, but data are needed for many species on exactly what viability testing schedule is desired. Viability tests using fewer seeds have been proposed but unfortunately are not widely utilized (30,31). Statistically based improvements in this area are known and should be developed and incorporated. Records of germination data for accessions must be orderly maintained for examination and interpretation by trained personnel. Often they are not readily available.

The recommendation from the IBPGR for retaining maximum diversity within an accession is to regenerate seed whenever germination drops about 15% below what was initially present before storage (26). The previous recommendation had been 5-10% below initial levels (53). Practical considerations of available labor, space, and time, however, often delay regeneration until viability has dropped 20 to 50%, or even much more from the original. For example, seed maintained accessions of potato held at IR-1 Potato Introduction Project are not regenerated until germination has dropped to below 50% of the original. Although this may not lead to loss of diversity in highly homozygous/uniform accessions, a highly heterozygous and heterogeneous accession will probably experience loss of genetic information. Loss of viability during storage is associated with mutational events, notably accumulation of chromosome damage and perhaps point mutations. Cells with chromosome change are usually selected against during growth of the seedling (diplontic selection), but point mutations may be passed to succeeding generations (90,91,95). Thus, genetic changes do occur during storage. Thus, regeneration phases should be done more timely.

Intertwined within the question of tolerable percent drop in viability before regeneration is the question of how many individuals are used to produce the next generation of seed (82). Minimizing regeneration cycles by using lower temperatures should reduce the rate of genetic drift in the population. The proposal to regenerate an accession whenever germination drops about 15% (as opposed to the more practical, 20-50% decrease), tends to increase the frequency of regeneration and associated costs (95,96,97). The balance between loss in diversity from delaying regeneration cycles and hence using seed samples with lower viability, and the loss due to genetic drift occurring from more frequent regeneration cycles if the 15% viability decrease is adopted is not easy to evaluate unless adequate characterization of diversity is available. Genetic drift problems could be reduced if larger numbers were grown out for regeneration, but practical considerations (e.g., space, manpower) usually limit the number of individuals used. Population genetics along with knowledge of rare allele frequencies are necessary for estimates of necessary numbers.

4. Cryogenic storage

At present cryogenic storage of seed requires both research (recalcitrant seed; large seeds) and development (orthodox seed). Where an adequate source of cryogen is available, cost estimates suggest that cryogenic seed storage is competitive with low temperature (-20°C) storage (106). A number of assumptions are made in these calculations, but if costs associated with regeneration cycles and germination testing are included, cryogenic storage appears less costly.

There are few procedural guidelines for routine storage in liquid nitrogen. Sample sizes should be similar to that used in cold temperature storage; however, seed size would affect numbers stored. Sample holders need to be evaluated. There are no guidelines for numbers of shoot-tips required for cryopreservation. LN refrigerators (where samples are stored) are standard units used in semen and microbial/animal cell storage system and should be useful for plant seed, pollen, and bud storage.

Cooling of orthodox seed and orthodox pollen requires no special equipment. Seed is merely placed into the LN storage refrigerator. Cooling of buds and shoot-tips, and perhaps recalcitrant seed, requires careful control and suitable equipment is available (54,115).

Effectiveness rests upon preservation of genetic diversity. Under conventional seed storage, regeneration is more frequent and some loss of diversity is likely. Maintenance of genetic diversity with cryogenic storage would appear to be more reliable, but needs confirming data.

Cryogenic storage of buds and shoot-tips for clonal lines requires considerable research before developmental studies are initiated. Few laboratories, however, are engaged in such work (117). A major scientific restraint is the genetic stability of regenerated plants. Virtually no information is available for either herbaceous or woody lines. As far as we are aware, no institute or program has routinely stored clonal germplasm under cryogenic conditions. Thus, no consensus or information on replicates, collection duplication, retrieval systems, distribution, etc. are available. Equipment for controlled cooling to LN is more expensive than that used for cooling seed and must be reliable. A source of cryogen must be available.

The genetic stability of materials exposed to cryogenic protocols has been questioned on two issues. The first is whether extremely long-term storage exposes samples to background levels of irradiation that over time could produce genetic change or lethality since DNA repair processes are not functioning at low temperatures (73). The second is pertinent to hydrated materials which are processed with cryoprotectants, i.e., whether the cryogenic protocol itself is mutagenic (20).

There are few data in higher plant systems for either issue. Projections from animal cells exposed to high levels of radiation while cryogenically stored suggest that lethality would occur only after several thousand years with background radiation levels (10,73). Clearly storage is not indefinite at low temperatures, but the results suggest a few centuries of storage may be a realistic period.

Microbes are the system of choice for examining mutagenesis of cryogenic protocols but no clear answer exists. A related process, freeze-drying, is mutagenic, but this is usually attributed to events during the drying phase and not cooling and thawing per se (7,108). Other studies suggest that DNA is damaged during cryogenic exposure (20). Notably, the use of some cryoprotectants allowed higher amounts of survival, and in these cases less DNA damage is observed. There are no comparable studies with cells or tissues from higher organisms; such information is needed.

The opinion, therefore, of most cryobiologists is that cryogenic storage is a safe proposition for retaining viability for many years. Cryogenic storage has been pragmatically used for microbes and for animal cultures, semen and embryos although the storage times required are usually only months to a few years (8). The American Type Culture Collection is now storing plant callus from diverse species in LN (5). The major problem for all hydrated tissues is in devising methods to cool tissues to these temperatures, retrieve and regrow them without greatly decreasing survival (68,118). As a contrast, dry, orthodox seeds easily survive low temperature exposure, but for both hydrated tissues and seeds, genetic stability with time must be demonstrated. A persistent problem in the cryogenic storage of in-vitro cultures is the maintenance of organization and induction of regeneration without (or with minimal) callusing or adventitious development.

5. Training

The storage and regeneration phases of germplasm preservation require suitably trained personnel for most positions. Implementation of preservation technologies in developed countries is not limited by training at the technical or clerical levels. Record keeping is largely computerized and training is readily available for most hardware/software systems. Personnel for field grow out for regeneration require basic knowledge of agronomy and horticulture, again usually readily available. Laboratory procedures differ considerably and specific technical training is necessary, and is often learned on the job. Seed technology, biochemical, and in vitro culture methods and techniques are demanding and several mechanisms serve to train the necessary personnel. Short courses are often available through industries and universities. For example, plant cell culture short courses are offered through the University of Tennessee and The Catholic University. Mississippi State, Iowa State and Oregon State Universities, among others, have courses in seed technology. Cold and cryogenic storage techniques for seed do not involve any difficult procedures and, as such, personnel can be trained on the job. Cryogenic storage of in vivo and in vitro buds is more demanding but training still usually occurs on the job.

Training at the technical level is with regard to methods and procedures, and is usually not directed toward germplasm preservation. Thus, job supervisors must relate methods and procedures to the tasks necessary to insure preservation.

The critical issue necessary for successful preservation is adequate training of genebank managers in all aspects of germplasm management. Training minimally consists of knowledge of crop biology, population biology, seed biology, storage technology, and equipment operation. Personnel should periodically be informed of new developments, ideas, and trends. Application of this knowledge to specific crops of concern is essential. Direct cohesive training mechanisms are lacking in the U.S. and minimal in other countries (55,58,59), although a number of universities offer courses on necessary subjects. The IBPGR considers lack of training in developing countries to be a major drawback to improving genetic conservation worldwide (59), and has helped support several courses of varying lengths on diverse topics (55,58,59). Preservation at genebanks worldwide is constrained by the observation "that many curators do not pay sufficient attention to management of the germplasm under their responsibility" and often "do not systematically keep accurate records, rejuvenate seed, verify collected material, nor regularly multiply and distribute collected material" (57). Clearly lack of dedication compounded by insufficient training can contribute to such problems.

III. Summary

The preceding emphasized the applications and evaluation of storage technologies for maintenance of genetic diversity within crop and wild species. Certain conclusions become evident.

A. Seed-Maintained Germplasm

1. Cold storage increases longevity in orthodox seed. Cryogenic storage should further increase longevity and should be evaluated for base seed collections. Seed used to regenerate active collections should be stored at temperatures lower than 5°C to maintain diversity. Regeneration schemes need to be critically evaluated to ensure adequate numbers are used.

2. The potential for new crop development is great and strategies for collection of genetic diversity of wild species must be assessed. Storage of many wild species could be as seed under cold storage. If the seed are orthodox, cryogenic storage could provide minimal maintenance until evaluations and use dictates an active collection. However additional research for cyrogenic storage is needed.

3. Many species with recalcitrant seed are vegetatively maintained because of seed storability problems. Research is needed in the basic physiology and biochemistry of recalcitrant seed. In-vitro storage is an effective supplemental system to maintain active collections and could also be utilized for a base collection of recalcitrant seed species.

4. Characterization and evaluation of seed germplasm, not limited to agronomic characteristics, are necessary before the effectiveness of storage and regeneration techniques can be evaluated.

B. Vegetatively-Maintained Germplasm

1. Germplasm is maintained vegetatively if discrete clones are required and/or if the time to reach reproductive capacity is very long (several

years). Certain other cases for clonal preservation occur, such as sterility in a desired line. Field preservation is common and for some materials is now combined with the use of in-vitro preservation.

2. Many aspects of clonal preservation have not been as well studied as seed preservation. Much research is needed for topics relating to grafting, floral induction, juvenile-mature transitions and control, genotypic stability, rooting ability, and hardiness, and how these physiological traits affect preservation strategies.

3. In-vitro preservation is a useful technology which needs research and development for application to clonal germplasm.

4. Cryogenic storage of shoot-tip and axillary buds from in-vivo and/or in-vitro plants has potential for long-term, base collection storage, but considerable research is needed before any development or implementation phase.

5. The concept of a base collection is not well developed for clonal materials. Seed and pollen populations together could be used for base collection storage of certain materials.

6. Characterization and evaluation of materials are needed to assess effectiveness of in-vivo, in-vitro, and cryogenic storage on maintaining genetic stability and genetic diversity in clonal lines.

IV. Objectives and Actions to Accomplish Objectives

The preceding conclusions emphasize that much remains to be done in improving germplasm preservation. To address this, we have identified three broad objectives along with positive actions to strengthen the total system. Three previous reports have made key observations and recommendations (41,42,79). Some have been implemented. Development of GRIN has improved information flow. Evaluation of germplasm for useful crop characteristics has received increased funding. Germplasm enhancement strategies are being initiated to make wild germplasm more useful to a breeder. Funds for a new NSSL are being considered and an ARS germplasm preservation research group has been established. However all aspects of current routine and research operations need to be critically assessed. The following 3 objectives are needed to improve germplasm preservation.

A. IMPROVE OVERALL PLANT GERMPLASM SYSTEM

The National Plant Germplasm System has evolved into a loosely coordinated network of activities of Federal, State, and Industry research units, of universities, and other organizations working together to introduce, catalog, evaluate, maintain, and distribute plant germplasm. The sum total of the collective efforts is to save and protect genetic materials of all plants of interest for future crop improvement, research, and other uses. The recent revival of interest in germplasm is credited to the 1970 corn leaf blight attack that caused a 15 percent loss in the U.S. corn crop. Interest continues to grow in all sectors of society for the preservation of germplasm, particularly of food crops.

1. Organize all plant germplasm activities within federal, state, and private sectors into the NPGS

The NPGS is a network of federal, state, and industrial organizations which has functioned well for food crops. ARS is the key agency in supplying leadership. Forest genetic resources and many wild species are not covered within this framework, instead being governed by the U.S. Forest Service and U.S. Fish and Wildlife Service respectively. Botanical gardens, arboreta, and other state units not presently included in the NPGS should be invited to participate. Storage problems and technologies are similar for all. A more coordinated system could facilitate cooperative research, utilize common facilities, avoid duplicated efforts, and enhance communication among the people necessary for successful preservation. Adequate administrative structure will be required to lead and coordinate diverse interests to accomplish established goals and objectives.

2. Ensure better communication among all germplasm collections by sponsoring periodical meetings and distributing informative newsletters

The complexity and diversity of issues within an expanded NPGS, advances in research and development, and effective management require information to be rapidly shared among units. The IBPGR has been effective in their overall mission, in part, because of effective communication. The NPGS lacks this. We encourage meetings as held in Ames, Iowa in June 1983 to be a regular mechanism for information exchange as well as the frequent publication of newsletters. (e.g., DIVERSITY and more informal equivalents). Better interactions with international groups and information about international activities are also needed. Better communication will enhance preservation by informing personnel of technological advances and allowing time for effective implementation.

3. Involve all components of the expanded NPGS in GRIN

The establishement of GRIN and CAC's improved various aspects of germplasm handling, collection, and evaluation. The system should be extended to allow all germplasm units, including the Forest Service, Fish and Wildlife

Service, botanic gardens and arboreta, state collections, industrial collections, to enter collection, evaluation, and cataloguing data. This will aid many users, especially of wild species and minor crops, in locating materials and in developing and implementing strategies for collection missions, evaluation, and preservation.

4. Develop closer cooperation with the IBPGR and other national and international programs

IBPGR is intimately involved in recommending, evaluating, and implementing preservation technologies. Their experience should be drawn upon for expanded efforts within the U.S. Closer cooperation among world genebanks and research groups definitely would aid preservation technologies.

B. IMPROVE GERMPLASM MANAGEMENT PROCEDURES

In the past 10 years significant new information on germplasm maintenance, collection and evaluation has been developed, in part due to the activities of the NPGS and IBPGR who have focussed on the various components of the system. It is now time to examine the present day-to-day operations, develop more stringent guidelines, and implement new knowledge to improve upon current procedures. The following are necessary:

1. Appoint a task force to examine existing procedures for storage and regeneration of plant germplasm

There have been few safeguards against loss of genetic diversity. No studies have systematically addressed problems of inadequate cataloguing procedures and records, inadequate regeneration procedures, inadequate drying, storage, or viability testing conditions, and of improper management. Most personnel are dedicated but many factors are often beyond control, yielding expedient actions which can erode diversity. A task force from all units of government, industry, and the international community must critically assess how day-to-day procedures affect preservation. Data on viability in storage of all collections should be pooled, made available, and evaluated. Suggested standard operating procedures should be recommended. When all procedures have been evaluated and improvements made, crop advisor committees (CAC) and regional technical committees could have responsibility for critically monitoring performance in a periodic fashion. CACs function for a given crop to evaluate national and international developments and provide overviews for future issues, particularly in breeding and genetics (78). Although they are concerned with germplasm issues (adequacy, evaluation, exploration), they do not now monitor performance of many routine factors necessary for preservation.

2. Establish strong guidelines for base collections of seed and establish base collections for clonal and other materials not presently having both active and base collections

The NSSL is the major U.S. genebank for base collections and a number of issues must be addressed by actions and funding. For example, additional storage space is needed (and is currently being examined). Not all active collections are represented in the base collection, especially for non-agricultural crops. Sample size in storage for many lines must be increased for adequate preservation.

The base collection concept for seed maintained crops is very useful and should be developed to a greater extent for clonal lines. Policy statements are needed. Storage of alternative propagules should be considered. The establishment of a base collection at a second site should also be considered to further insure preservation of diversity.

3. Develop effective training programs for key personnel within germplasm collections

Each technology requires trained, dedicated people to operate the facility and ensure that germplasm is maintained. The curator has the key role of implementing actions in operation and management, and in recognizing and effectively dealing with problems. Effective training is necessary to perform these functions. New technologies and research findings must be conveyed to personnel such that they can be part of evaluation and preservation strategies.

4. Increase and broaden effort on germplasm characterization and evaluation

The effectiveness of technologies in preserving genetic diversity can only be critically assessed after detailed characterization and evaluation of accessions are performed. Maintenance of viability is no assurance that diversity has been preserved. The evaluations necessary are complex and must utilize techniques and methods from basic research.

5. Develop collecting strategies which allow sampling of all desired species

The best storage technologies can only preserve what has been collected. Emphasis on the collection of germplasm is desirable, but priorities must be established to use limited funds. Coordination with IBPGR is necessary to avoid duplicate collecting missions, to minimize omissions, and to share collections.

C. IMPROVE AND EXPAND BASIC RESEARCH ON PLANT GERMPLASM PRESERVATION

As summarized above there are many questions which need answers and these can and should be researched. Effective collection, maintenance, evaluation, enhancement, and preservation technologies are all dependent on a sound program of research. Future success in maintaining and utilizing our vanishing genetic resources will hinge upon the support given to research. A broader spectrum of research activities is needed to obtain the answers to these questions and achieve our national goals in the area of germplasm resources.

1. Develop theories and strategies for intermediate and long-term preservation of all plant germplasm

a. Determine causes of loss of viability and genetic diversity in seeds, pollen, and vegetative propagules.

b. Develop cryopreservation techniques and determine effects on physiology and genetics of plant germplasm.

c. Develop *in-vitro* short- and medium-term preservation techniques for clonal materials.

d. Investigate alternative strategies for germplasm preservation, such as utilizing isolated RNA and DNA.

In the past germplasm storage has essentially referred to seed storage and more specifically to orthodox seed storage. It is becoming increasingly apparent that new techniques for storage of non-traditional forms of germplasm such as recalcitrant seeds, pollen, apical and axillary meristems, cell cultures, etc. are needed. Although cryogenic storage has been used for several years in the animal kingdom, it is only recently being investigated for plant germplasm preservation. *In-vitro* storage methods need to be developed for the diverse array of clonal materials. Basic knowledge is needed on the mechanism of cell deterioration and its avoidance or repair in order to ensure maintenance of the genetic integrity of our plant germplasm.

Alternatively, new methods for storage of naked DNA and RNA and possible recovery of DNA from dead cells could lead to a whole new concept in germplasm preservation. Caution must be exercised here such that limited funds are not disproportionately channeled into this high-technology area. Genetic preservation is the key issue and existing technologies with their proven value and importance must receive adequate funds before more speculative approaches are heavily funded. Considerable discussion on the projected value of these isolated gene techniques for germplasm preservation must precede funding.

This research should occur at a number of locations dependent on the crops of interest in a geographical area and on the skills and expertise available. Although interactions are encouraged, storage strategies would benefit from diverse alternative approaches.

2. Develop biochemical, genetic, and physiological techniques for characterizing and evaluating plant germplasm at the gene, genome, and population levels

Germplasm preservation implies the maintenance of genetic integrity of the plant material. However, in most cases germplasm preservation is monitored solely by means of a viability test. Methods for evaluation of the genetic integrity of germplasm and its response to different storage regimes will ensure the availability of the full range of genetic variation for a given species.

3. Investigate genetic, cytogenetic, population genetic relationships between wild species and domestic relatives

In order to fully understand the range of diversity for a given species more information is needed on such factors as chromosome number and behavior, degree of relatedness among species, crossability, incompatibility factors and geographical distribution. In many cases wild relatives of domestic species have not been studied for factors as basic as germination and response to storage.

All the above actions require a coordinated system. The federal government has largely been responsible for organization and support of germplasm efforts. Continued federal leadership is necessary because of the national importance of germplasm, the international interactions required for collecting and operation of world genebanks, and the close coordination among federal, state, and private units. The actions themselves can be performed by the Federal, state, and/or private units. The planning of cooperative programs will utilize people from each level. Cooperative efforts have been very productive in the past and should function well here if a sound plan is formulated by the leaders. "Networking" has been successful in various national and international agricultural issues and the principles could be applied here to coordinate the diverse national groups to solve the common issues. The consequences of taking actions would be to have a dynamic system providing better preservation through examining and evaluating new methods and by constantly monitoring performance of the component groups.

The NPGS concept is vital to preservation because of policy developments, coordination, and funding support. Some of the weaknesses in the NPGS (41,42,79) have been corrected but others remain. The crucial issue is to develop strong leadership and define mechanisms for implementing recommendations from task forces. State and private institutions will always be autonomous, but they must be willing to support procedural recommendations for improvement of the NPGS if they are to be effective members. Funding support from the federal sector should facilitate this.

V. Mechanisms to Encourage Action on Objectives

A. Germplasm Preservation Act

Because the whole issue of germplasm is larger than just agriculture and, in fact, involves several cabinet departments, Congress may wish to establish a bureau, commission, agency, etc., (for example similar to NASA), to oversee all aspects of germplasm collection, preservation and utilization. Alternatively this structure could be as an interagency unit headquartered within ARS, providing appropriate concerns for all germplasm issues are addressed. Discussions would decide the best structure. The major point is that coordination and action are facilitated and given importance. This new structure could include animal as well as plant germplasm. An act similar to the Endangered Species Act may serve as a focal point and could stimulate both public and private entities towards increased awareness and actions of the importance of germplasm in maintaining our world leadership in agriculture, forestry, and related areas.

B. Increased Financial Support

1. Existing programs

There is a critical need for additional funding for all aspects of the germplasm program. This funding is necessary for the application and upgrading of preservation technologies to existing active and base collections, and for stimulating new directions in research on germplasm problems. The NSSL, regional plant introduction stations, and the newly established clonal repositories need additional staffing and financial support to adequately preserve genetic diversity.

2. Competitive grants for germplasm

In order to broaden research efforts and bring in additional scientific expertise from other sectors, funding should be provided for competitive grants in the area of germplasm resources. These grants could be admininstered through the existing USDA competitive grants program or through the agency described above, and should support research related to germplasm collection, evaluation, maintenance, and utilization for both plants and animals.

APPENDIX A: MAJOR CROP GROUPINGS

1. Cereals and grain legumes

Cereals and grain legumes are our most important staple food crops and considerable effort is expended on improvement strategies (54,55,58,59,72). This has led to extensive collections of cultivars and land races (86). Germplasm is stored as seed at cold temperatures. Cereals are the crops best evaluated for known agronomic traits, but evaluation is variable and usually minimal among different collections. The extent of duplication within a collection is usually unknown; duplication among different collections is sometimes extensive.

2. Forages: legumes and grasses

Many different species are utilized as forages in different parts of the world, and many countries now have working and base collections (27). Most are stored as seed, but some forage grasses are maintained as clones. Evaluation among the many collections world-wide is minimal, but where done, is usually for agronomic characters of advanced lines.

3. Vegetable crops

This category comprises diverse species, including leafy vegetables (e.g., lettuce, endive), some legumes, cole crops, herbs, Solanaceous crops (tomatoes, peppers), cucurbits (squash, cucumbers), beets, onions, and celery. Most germplasm is stored as seed. Some materials are stored as vegetative plants (i.e., French tarragon, garlic) because of sterility barriers. These plants are maintained in greenhouses or fields and are divided for propagation. World-wide a number of genebanks occur and more are being designated (58,59,110). Less effort has been expended collecting these resources compared to cereals/legumes. Collections among the species varies considerably.

4. Tree crops: forest and timber

Natural wild stands are utilized and managed, but in many areas single species stands of superior types are grown. Within natural stands, considerable diversity exists for tree improvement (107). Tree breeding, however, requires long periods of time before line evaluation. Vegetative multiplication of desired individuals is sometimes feasible, but many stands are generated from seeds.

Seeds from many of these species are orthodox and can store easily; however, when an active collection is depleted of seed, regeneration is very difficult because of the long time required to grow a seed to the mature, flowering plant. Seeds from some species are recalcitrant (22). Seeds may serve as a suitable base collection for species with orthodox seed but often vegetative plants are both the base and active collections. Recalcitrant species seed stored with currently available methods cannot serve as a base collection. Vegetative plant maintenance is, thus, used for preservation but more satisfactory strategies are necessary. Long-term preservation could utilize combinations of pollen, seed, and vegetative materials, the longevities of which theoretically could be lengthened by low temperature treatments. Preservation efforts require research on understanding and manipulating juvenile-mature phase transitions.

5. Root and tuber crops

This group contains species whose germplasm is maintained as seed alone (e.g., carrots, sugarbeet), and as both clones and seed (e.g., potato, sweet potato, yams, cassava). Seed is usually stored between 5°C and -20°C. Clonal lines usually are grown out each year. Tubers and roots can be stored about 1 year at 2 to 10°C.

In-vitro techniques for virus-tested plantlets derived from meristem-tips and buds of clonal lines are developing rapidly for potato (75), sweet potato (51), yams (3), and cassava (94). Potato and cassava *in-vitro* plantlet storage at low temperatures and under minimal-medium conditions is being investigated at the International Potato Center (CIP), the U.S. Inter-regional Potato Introduction Project (IR-1), and Centro Internacional de Agricultura Tropical (CIAT).

6. Temperate fruits and nuts

Stone fruit and nuts (peaches, plums, cherries, almonds), pome fruit (apples, pears), brambles (raspberries, blackberries), and berry fruits (grapes, strawberries) are some common members in this category (119). Diversity from centers of origin has disappeared rapidly and has been inadequately collected (16). Vegetatively maintained lines are usually collected as vegetative cuttings, whereas seed propagated lines are collected as either seed or cuttings. Collections exist in many countries and usually consist of cultivars and rarely related wild species. Orchards (field genebanks) are the main storage system. Scions can be stored for several months at -1°C to -3°C (70). The establishment of the U.S. Germplasm Repositories for fruit and nut crops should allow better preservation as well as facilitate germplasm exchange among genebanks world-wide.

In-vitro culture promises to be of value in propagating and storing fruit and nut germplasm in a disease-free state. Methods for *in-vitro* culture of many members have been established (24,71,102,120). Geneotypes are stable if shoot-tips are the initial explant and plant regeneration avoids a callus stage and/or adventitious development. Current use supplements orchard maintenance, but *in-vitro* culture could form part of a base collection.

Pollen and seed storage are underutilized and should be considered for portions of both active and base collections (1).

7. Tropical fruits and nuts

Major tropical fruit species include avocado, banana, breadfruit, cashew, citrus, date, fig, mango, papaya, pineapple, and others less well known in the U.S. The IBPGR has compiled current holdings of genebanks throughout the world (45). The numbers of accessions in each genebank varies considerably but most are stored as plants with cuttings, budwood, suckers, and/or seed being distributed. Budwood and seeds are held under cold storage. Where listed, often only a few individuals exist per accession. Few details exist on duplication of materials within and among collections. Compared to seed crops or vegetables, accessions for most tropical fruit are few in number. *In-vitro* storage is being examined but is currently supplemental to *in-vivo* storage.

8. Ornamental species

These diverse species can be divided into annual flowering plants, herbaceous perennials, and woody perennials. Collecting expeditions and establishment of genebanks have not been extensive, and many consist of efforts of informal groups of individuals interested in particular species. Germplasm of annual flowering species is maintained as seed under conventional storage conditions. World genebanks have not been designated although from some flower species seeds are within base collections (e.g., NSSL) (13). Collections are usually maintained by individual university researchers interested in improvement, and perhaps more so by industry for their improvement programs. Some cultivars after development are maintained clonally as vegetative plants and divided or multiplied *in-vitro* for distribution. Rapid, large number multiplication and rooting is presently the most economical and extensive use of in-vitro culture (52).

Herbaceous and woody perennials are maintained clonally in botanical gardens and arboreta. Collections usually have only a few genotypes of each species. Information is sparse on the genetic diversity available for a species, materials often being stored in distant locations. In-vitro culture would be very useful for supplementary preservation strategies.

9. New crops

Only about 100 of the some 350,000 known plant species are used as crops for food and fiber in the United States. World-wide about 300 species are used (88). Thus, over time there is good potential for developing new domestications for food and fiber as well as for chemical sources, energy sources, and medicinal compounds. Work evaluating species for potential use in several areas has been done by ARS-USDA. Jojoba for oil and amarantha for grain and vegetables are two illustrations of new crops slowly being developed in U.S. agriculture; other such as the winged bean and taro are being developed in the tropics (76). Considerable expansion of federal, state, and industry support is needed if such materials are to be adequately researched and developed as new crops (25). If support occurs, preservation strategies of new materials must be examined.

Collecting the genetic and species diversity for a new crop usually is not done until after some modicum of potential is recognized. Botanic gardens and arboreta have been involved for many years in the collecting and preserving of wild species (34). This effort is becoming better coordinated. Existing preservation technologies should allow adequate storage if questions such as seed germination and regeneration procedures are addressed. Some of these materials might be stored solely under cryogenic conditions until use warrants establishment of active collections.

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TABLE 1: APPLICATION OF STORAGE TECHNOLOGIES TO GENEBANKS OF DIFFERENT CROP GROUPS

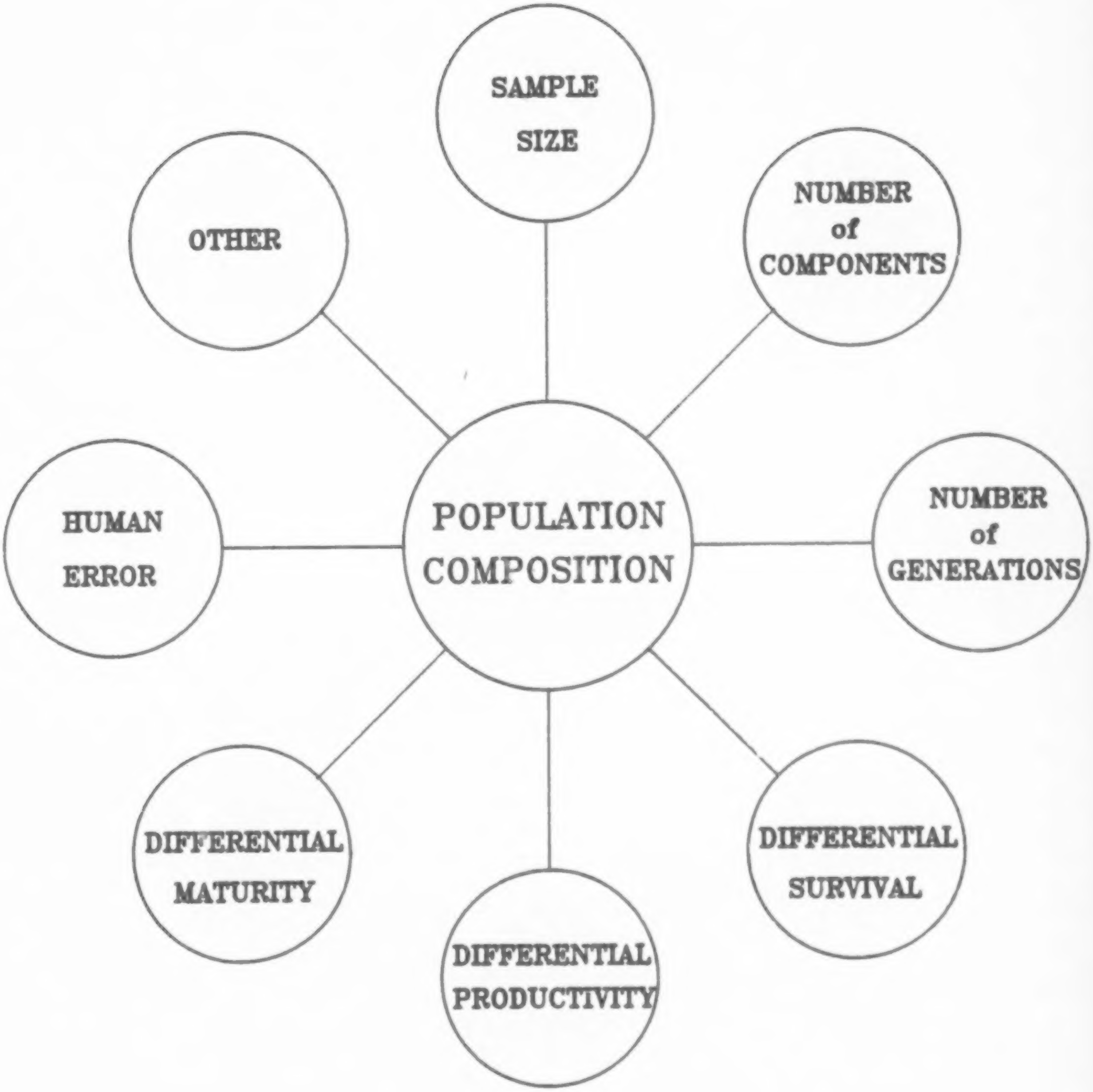
Germplasm Category	Representative Examples	Propagule Stored	Storage Technology				Collections Available	
			In-Vivo	In-Vitro	Cool Temp.	Cryogenic	Base	Active
Cereals & Grain Legumes	Wheat, Oats, Barley, Rice Soybeans	Seeds			X[1]	R[2]	X	X
Forage Legumes & Grasses	Alfalfa, Clover, Bromegrass, Orchardgrass	Seeds			X	R	X	X
		Plants	X	R				X
Vegetable Crops	Tomato, Bean, Onion, Carrot, Lettuce	Seeds			X	R	X	X
		Plants	X	R		R*[3]		X
Tree Crops Forest Timber	Pines, Firs Hardwoods	Seeds			X	R	X(?)	
		Plants	X				X	X
Root & Tuber Crops	Potato, Sweet Potato, Yams	Seeds			X		X	X
		Plants	X	X,R		R,R*		
Temperate Fruit & Nuts	Strawberry, Peach, Apple, Raspberry, Grapes	Seeds			X	R	X(?)	X
		Plants	X	X,R		R,R*		
Tropical Fruit & Nuts	Avocado, Banana, Citrus, Date, Papaya	Seeds			X	R		X
		Plants	X	R		R*		X
Ornamental Species	Carnation, Zinnia, Lilac, Rhododendron	Seeds			X	R	X	X
		Plants	X	X				X
New Crops	Jojoba, Amaranth, Chemurgic Plants	Seeds			X	R	X	X
		Plants	X	X				X

[1] X: currently in use

[2] R: under research and development phase

[3] R*: under research and development using buds/shoot-tips from in-vitro plants

Figure 1: Factors which can affect population composition of a germplasm accession. The category "other" could include, but is not limited to, disease epidemics, insect attacks, and adverse weather conditions.


SAMPLE SIZE
OTHER
NUMBER of COMPONENTS
HUMAN ERROR
POPULATION COMPOSITION
NUMBER of GENERATIONS
DIFFERENTIAL MATURITY
DIFFERENTIAL SURVIVAL
DIFFERENTIAL PRODUCTIVITY

Contract Paper No. 6

Technologies to Evaluate and Characterize Plant Germplasm

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TECHNOLOGIES TO CHARACTERIZE AND EVALUATE
PLANT GERMPLASM

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October, 1985

ABSTRACT

A variety of techniques and methodologies have been and are being used to characterize and evalu ate plant germplasm. In the past, primary emphasis has been placed on morphological or other traditional agronomic characters for these purposes. During the past twenty-five years considerable technological advances have been made that can be used to rapidly and efficiently screen large numbers of individuals for particular characteristics. However, in most cases the application of the available technology to germplasm evaluation has lagged behind its development and application in other fields. Thus, the technology which could permit significant improvements in germplasm evaluation already exists. What is currently required is the encouragement to apply this technology to germplasm evaluation.

At present, evaluation of genetic diversity in plant collections consists of a visual observation of selected morphological and agronomic characters during grow-outs or seed increases at primary storage centers, such as the Regional Plant Introduction Stations. This primary screen is usually followed in major crops by more intense studies, at specialized laboratories, of certain physiological and biochemical traits. Although the new methodologies can not replace the information obtained during the primary evaluation, it appears that several technologies could be applied during this initial evaluation in order to obtain a much better characterization of the genetic diversity present within and among accessions.

For instance, salinity tolerance and virus resistance appear to be characters than can be rapidly assessed in young plants by recently developed methods. Allozyme characterization, although not a new approach, now could be used on a routine basis for directly evaluating genetic diversity during the primary screen. Similarly, analysis of flavonoids or other suitable phytochemical compounds could be performed at this time because the development of new instrumentation and procedures have greatly simplified phytochemical analysis.

One technique with great potential for evaluating genetic diversity is analysis of restriction fragment length polymorphism. Unfortunately, several critical improvements in methodology are necessary before this technique can be used as a general tool for germplasm evaluation.

Institutions and government organizations can contribute to the development of more efficient germplasm evaluation by encouraging, through grants and training programs, closer ties between the primary evaluation centers and research laboratories possessing expertise in the desired technology. Development of a more thorough screen for genetic diversity is of immediate concern because such information is particularly useful in identifying geographical regions that should be more extensively sampled. Increased attention to quality control of the collected material and greater emphasis on obtaining data from outside users of the germplasm collections is also recommended.

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Appendix I

INTRODUCTION

Plant germplasm evaluation has been intimately associated with agriculture ever since Neolithic man first began to collect seed and to grow crops. When selecting seed for planting, these early farmers often chose the larger seed or seed from plants which had remained healthy throughout the growing season. Whether an active or passive process, this selection activity produced most of the domesticated lines available 100 years ago. Within the last century, our understanding of the genetic material and the mechanisms of inheritance has increased tremendously. This knowledge has permitted more efficient introgression of useful traits discovered in exotic germplasm and the selection of combinations of characters in breeding lines (Allard, 1960; Dubinin, 1965; Frey, 1976; Rick, 1974).

Today, the evaluation of germplasm is more organized and can be much more sophisticated than the selection activities of early farmers; however the process retains the same basic goal: to identify and isolate naturally occurring variation which may be of value to science and agriculture. We know that such variation must have a genetic basis (i.e., must be capable of being passed on to future generations) in order for it to be of much use in breeding programs. Furthermore, we have recognized that it is difficult to predict what variation will be useful in the future, and most authorities now recommend preserving as much natural biological diversity as possible (Frankel, 1974; Harlan, 1975; Oldfield, 1984). Attempts to generate new diversity by treatment with mutagens have met with partial success, but many important traits appear to be difficult or impossible to induce by mutation. Attempts to select new phenotypes in the absence of genetic variation (Lysenko, 1946) have proved fruitless. Moreover, the discovery of important biochemical products (Kreig, 1964; Swain, 1972; Balandrin, et al., 1985) or resistance to specific diseases (Sears, 1956; Kimber, 1967; Riley et al., 1968) in plant introductions which otherwise appeared to be very poor candidates for cultivation has occurred so often that such cases can be regarded as the rule rather than the exception. Thus, a critical initial step in the evaluation of germplasm is to assess the extent of genetic diversity present within and among accessions. Ideally, all the genetic diversity, although not necessarily all combinations of this diversity, present in wild populations or breeding stock should be represented in germplasm collections. Practically, this goal is impossible to attain, but it does serve as a reference point against which the completeness of a collection may be measured.

Although germplasm collection and evaluation has been handled in the United States on a organized basis for nearly a century, a major commitment by Congress to support these activities occurred only after World War II with the passage of the Research and Marketing Act of 1946 (Public Law 733). This Act alloted funds for cooperative research between two or more State Agricultural Experiment Stations or between the Department of Agriculture and State Agricultural Experiment Stations. One of the first projects proposed under this Act was a national program for the introduction and testing of new plants. This proposal eventually resulted in the establishment of four Regional Projects (NC-7, NE-9, S-9 and W-6) which covered the evaluation of nearly all seed-propagated plants brought into the United States (Burgess, 1971).

The post-war preeminence of this country resulted in the plant germplasm evaluation program in the United States becoming the predominant undertaking of this sort in the world. Most other countries interested in germplasm evaluation appear to have developed programs similar to that in the United States. It is therefore convenient to use the program in this country as a model to describe current methods in germplasm evaluation.

In general, the initial evaluation of a plant introduction, after any necessary quarantine procedures, is performed at one of the four Regional Plant Introduction Stations or at research centers where national programs on a particular crop exist (Hyland, 1970). National research centers include those for cotton (College Station, Texas), soybeans (Urbana, Illinois), sugarcane (Cane Point, Florida), and small grains (Beltsville, Maryland). Each Plant Introduction Station has responsibility for several important crops and may also screen related minor crops and other species of interest. Before an accession is sent to a Regional Plant Introduction Station or research center, it is usually given a Plant Introduction (P.I.) number at the Department of Agriculture offices in Washington D.C. At this time, data obtained by the field collector regarding place collected, local name, Latin name, and particulars of the collection locality (elevation, soil type, surrounding vegetation, etc.) are transcribed on to a permanent record. This information constitutes the "passport data" of an accession and is compiled in Inventory books available at all agricultural experiment stations and in the libraries of land grant colleges.

To breeders and growers "evaluation" often means determining the yield and economic value of a particular cultivar or breeding line (Oyervidas-Garcias, et al., 1985; Makus, 1984). However, such evaluation is not appropriate for germplasm collections, for introduced lines, at least in the United States, will nearly always perform poorly compared to indigenous cultivars. Instead, evaluation of P. I. lines is usually for particular characters such as salt tolerance (Shannon and McCreight, 1984), cold tolerance (Erskin et al., 1981) or disease resistance (Provvidenti, 1982). Even this "narrow" evaluation is usually beyond the functions performed at the Regional Plant Introduction Station where time and resource limitations permit merely a superficial characterization of most accessions. The traits analyzed at these Stations are necessarily relatively easy to score and few in number. Responsibility for which traits are scored in a particular crop is supposed to rest with the respective crop advisory committee, thereby reflecting the need of the breeders; however, in many instances these committees have not decided on a specific list of descriptors, and the selection of the descriptions thus becomes the responsibility of the Station personnel.

The list of traits currently being evaluated for pea accessions is included as Appendix I, and although other crops are evaluated for a different set of characters, those for pea may serve as a general example for most crops. Thirty descriptors, most of which are morphological characters, form the primary data obtained in an evaluation of a pea accession (Appendix I). The 'Plant Introduction Number' is that assigned in Washington D.C. The 'origin' is the institution or country from which the sample was received, not necessarily the country from which the seed originated. Thus it is possible to have a sample of a Middle Eastern cultivar, which had been sent to Sweden and Japan, enter the Plant Introduction system as two different accessions, one from Japan and the other from Sweden. This problem may be overcome by going to the original collection number. However, breeders and other users of P.I. collections rarely make this extra effort, and occasionally the collection number is no longer available.

The remaining morphological descriptors used for pea are based on genetic variation already known to exist. Many are characters with a simple genetic basis (Blixt, 1974). The presence or absence of anthocyanins, for instance, is usually determined by a single locus, A. This trait is of particular interest to breeders because pea lines lacking anthocyanins make superior processing types, and virtually all commercial cultivars lack anthocyanins. Other traits such as the presence of pigment on the testa or hilum are not as crucial for breeders but make convenient genetic markers. The categories 3, 4, 5, 6, 11, 12, 13, 14, 15, 19, 20, 22, 27, 28, and 29 are polygenic traits requiring measurements or observations by trained personnel. Results of these evaluations are published by each Station in the form of an Annual Report, and all information available on each accession is also being entered into computer memory banks which can be accessed through the national germplasm informatin system (GRIN).

Except for occasional notes regarding unique characters or special projects undertaken by certain staff members no further evaluation is performed at Plant Introduction Stations. Additional characterization and evaluation is accomplished by plant breeders and other researchers in academia or private enterprise. Usually, such evaluations involve the screening of a limited set of material for relatively few traits. A phytochemist may concentrate on the alkaloids present in 100 different accessions, while a breeder will screen the same accessions for cold tolerance. These studies represent a very important part of the evaluation of accessions, for they are performed by individuals with a particular expertise and represent a considerable investment of time and money. Unfortunately, in many instances the results of such surveys are never returned to the Plant Introduction Station nor published in any other format available to the general public. Such surveys cannot be considered 'evaluation' in the strict sense because the results are unavailable, except to a very few individuals. Thus, the study would have to be repeated by another group wanting the same information. In cases where an outside investigator does return a report to the Regional Plant Introduction Station, such information is included in the Annual Report.

This tiered system for plant evaluation, with the Regional Plant Introduction Stations acting as a first screen and information distribution center and more detailed studies being done by academia and private enterprise, has worked relatively well in crops for which funds exist to support the additional studies outside the four Regional Plant Introduction Stations. Germplasm collections of secondary crops such as onions have been largely ignored, except for periodic seed increases and occasional studies by small research groups.

Work in other countries has provided a major proportion of the evaluation of genetic variation in several crops. Excellent studies on rice have been performed in Japan (Endo and Morishma, 1983) and the Phillipines (Chang, 1970, 1976, 1981). Most of the studies on chickpea have been done in India or the Middle East (Singh et al., 1984). However many of the evaluations of minor crops (e. g., Arora et al., 1982; Weltzier, 1982) do not go beyond the level of a primary evaluation as exemplified by the screening done at the Regional Plant Introduction Stations. An important organization at the international level is the International Board for Plant Genetic Resources (IBPGR), associated with Food and Agriculture Organization of the United Nations. This board supports many activities related to germplasm collection and evaluation, including the publication of the periodical "Plant Genetic Resources Newsletter" which provides an international forum for reporting the results of collecting trips and germplasm surveys.

CURRENT AND EMERGING TECHNOLOGIES

Three primary areas of germplasm characterization which have augmented by recent technological advances are:

1. Botanical and agronomic characterization.
2. Phytochemical screening of secondary products.
3. Electrophoretic determination of genetic diversity.

Each of these areas will be discussed separately.

1. BOTANICAL AND AGRONOMIC CHARACTERIZATION PROCEDURES

Botanical and agronomic characters are probably the traits of greatest interest to those using germplasm collections. Characters such as heat tolerance, photosynthetic capacity, disease resistance and habit are important aspects of any crop, and information on these traits is used not only by breeders but also by physiologists, pathologists, biochemists and geneticists. The set of characters that could prove useful to these scientists is so diverse that virtually any and all advances in technology could be applicable. The type of crops currently economical to grow in the United States are very different than those useful to farmers in Ethiopia or Brazil, and almost certainly other crops will be found to be better adapted to artificial environments under the oceans or in space. It would be impossible to describe all technologies that could or will be important in germplasm assessment; however several technologies will almost certainly be prominent in the primary evaluation process because they allow a rapid method for screening large numbers of individual plants. These technologies include analysis of plant properties using controlled environments, computer-aided analyses of plant morphology, scanning electron microscopy, and immunological methods for assessing disease resistance.

Large controlled environment facilities, such as the phytotrons at the Southeastern Plant Environment Laboratory (NCSU Phytotron) or the Duke University Phytotron, permit the growth of many plants under carefully monitored conditions. Although environment chambers do not represent a new technology, they are becoming increasingly complex, for the study of many important phenomena in plants requires that the plants be grown under specific and reproducable conditions (Mann et al., 1985; Baysdorfer and Bassham, 1985; Dominy and Heath, 1985; Eskins et al., 1985). The availability of such facilities enable breeders and other plant scientists to conveniently and reliably screen introductions for different environmental stresses.

Computer-aided analysis such as quantification of leaf area (Newell, 1985), the study of growth responses (Jaffe et al., 1985) and even data collections (Gilreath, 1985) should provide new characters to score or increase the efficiency of the characterization and evaluation process. The polygenic nature of the traits measured would still present a problem, but computer analysis may be able to divide the measurements into simple characters to partially circumvent this difficulty. Similar arguments may be made concerning characters visualized under the scanning electron microscope. Interesting and, perhaps, useful variation has been revealed by scanning electron microscopy (French, 1985), and computer analysis of appropriate traits could provide substantial data on genetic variability in a population.

Enzyme-linked immunosorbent assay (ELISA) has been used to screen plants for the presence of viral proteins (Hill, et al., 1981; Van Regenmortel and Burckard, 1980). The technique is rapid and can be used to assay many plants simultaneously. Plants which permit viral infection to become systemic without exhibiting symptoms of the disease can be differentiated from plants that localize the infection. The importance of disease resistance in modern cultivars will probably dictate the screening of most accessions for resistance to many diseases, so that relatively rapid and simple procedures for such analyses would be desirable.

2. PHYTOCHEMICAL SCREENING OF SECONDARY PLANT PRODUCTS

Pytochemical screening of plant germplasm has been an activity of humans since before the time of the ancient Romans. However, during the past twenty-five years, phytochemistry has emerged as a distinct discipline, somewhat intermediate between natural product organic chemistry and plant biochemistry. Phytochemical screening is that branch of phytochemistry that deals with the natural distribution of the variety of organic compounds that are synthesized and accumulated by plants. Historically, a variety of micromolecular compounds have been used to characterize plant germplasm. These include, but are not limited to, phenolics [e.g., flavonoids (flavones and flavonols), anthocyanins, xanthones, stilbenes, quinone pigments], terpenoids (e.g., essential oils, gibberellins, steroids, carotenoids), organic acids, lipids, and related compounds [e.g., Krebs cycle acids (i.e., citric, malic, etc), stearic acid, oleic acid, glycolipids, polyacetylenes, glucosinolates (i.e., mustard oil glucosides)], nitrogen compounds [e.g., amino acids (including non-protein amino acids), alkaloids, cyanogenic glycosides, cytokinins, and the chlorophylls), and sugars and their derivatives (e.g., monosaccharides, oligosaccharides, sugar alcohols, and cyclitols). Choice of which particular class of compounds is to be utilized for characterization generally is governed by the specific plant group under consideration. For example, flavonoids are of almost universal occurrence in the vegetative and floral tissues of higher plants, whereas polyacetylenes occur regularly only in five families of angiosperms (Campanulaceae, Compositae, Araliaceae, Umbelliferae, and Pittosporaceae) and glucosinolates occur primarily in the cabbage family (Brassicaceae) (Harborne and Turner, 1984). Phytochemical screening has been and, most likely, will continue to be the method of choice for chemical characterization of plant germplasm because of the range and number of micromolecular compounds is so large (e.g., there are over 5500 known plant alkaloids) and the distribution of each particular class of compounds is so variable (i.e., variation exists at every taxonomic level; e.g., family to cultivar). In addition, phytochemical methods have been aided enormously by technological developments in the areas of rapid and accurate methods of screening plants for particular compounds.

In essence, the basic rationale for phytochemical screening, with respect to characterization of plant germplasm, is that particular classes of compounds tend to have restricted distributions within specific plant taxa, be they genera, species, or infraspecific groups (e.g., botanical varieties or cultivars). In other words, a specific taxon can be characterized (i.e., recognized) by its chemical constituents if those constituents are restricted to the taxon in question. This type of data is of particular use in establishing the identity of a specific germplasm line and for determining genetic, as well as phylogenetic, relationships among groups of taxa (Young and Seigler, 1981). These latter types of data are often of significant importance in establishing potentially new sources of genetic material from wild populatons that could be used in germplasm improvement programs.

With respect to germplasm evaluation, phytochemical screening is most applicable when it is a particular compound that adds or detracts from the general usefulness of a germplasm line. For example, evaluating the insecticidal properties of specific plants (e.g., Pachyrhizus erosus, the Yam Bean); determining levels of biologically active compounds, such as podophyllotoxin in Podophyllum and related taxa; evaluatng levels of the limiting amino acids in, for example, legume crops; or determining levels of toxic principles in specific germplasm lines (e.g., non-protein amino acids in legumes; Bell, 1972, 1978; Rosenthall, 1982).

The basic methods of phytochemical screening are similar, no matter what particular class of compound is considered, and involve three general processes: extraction, isolation (i.e., separation and purification), and identification (Harborne, 1973). Extraction techniques are fairly straight forward and often rather simple. Plant materials typically are homogenized in a blender or tissue grinder in aqueous alcohol to achieve exhaustive extraction. Initial extracts can be further fractionated, after evaporation of the alcohol, with successive extractions using hexane and/or chloroform (to separate lipids and terpenoids) followed by alcohol and/or ethyl acetate (for more polar compounds).

Conventional methods for the separation and purification of phytochemicals have utilized one or a combination of four chromatographic techniques: paper chromatography (PC), thin layer chromatography (TLC), column chromatography (CC), and gas liquid chromatography (GLC). Choice of a particular isolation technique depends largely upon the solubility properties and volatilities of the compounds being separated. PC generally is used for water soluble compounds (e.g., carbohydrates, amino acids, organic acids, phenolics); TLC for separating lipids, steroids, carotenoids, and chlorophylls; for non-volatile compounds, CC is a very useful technique for preliminary purification or for preparative scale separation of large quantities of compounds from crude plant extracts (polyamide, silica gel, and sephadex gel are the most widely used column packing materials); GLC is used primarily for separation of volatile compounds (e.g., fatty acids, hydrocarbons, terpenoids, and sulphur compounds). Most often, a combination of two or more of the above techniques yields the best results. Of course, when dealing with compounds that are charged (e.g., amino acids, some alkaloids, organic acids), paper and/or starch or acrylamide gel electrophoresis can be used in the isolation process.

During the past ten years the major technological advance in the area of separation and purification of complex mixtures of organic compounds has been the development of high-performance liquid chromatography (HPLC). HPLC is, perhaps, the most useful technique for the isolation of organic substances, and the range of compounds that have been successfully separated using HPLC continues to expand at a very rapid rate. In addition, there has been a dramatic increase in the scope and versatility of HPLC due primarily to improvements in instrumentation and the efficiency and nature of column packing materials (Hostettmann, 1982). The primary advantages of HPLC versus other more conventional chromatographic techniques are that it is relatively fast, extremely reproducible, and can be applied at both the analytical and preparative scales. Major disadvantages of HPLC are that it does require an initial investment of time to develop appropriate solvent regimes for the complete separation of complex mixtures of compounds, and often quite different solvent combinations are required for different classes of compounds (although, once established these solvent systems can be used routinely), and the cost of the instrumentation. For example, a typical gradient HPLC system costs between $25,000-30,000; a preparative scale column over $800 (compared to about $100 for 100 sheets of chromatographic paper). There is, however, little doubt that under the right circumstances, HPLC could become the method of choice for the isolation of many groups of plant compounds, and for routine qualitative and quantitative phytochemical screening of plant germplasm.

A technique that has not found wide use in the isolation of phytochemical compounds but does have significant potential, particularly when dealing with phenolics, is droplet counter-current chromatography (DCCC) (Tanimura et al., 1970). This technique is very helpful in the preparative-scale separation of polar compounds (e.g., flavonol glycosides). Recently, Hostsettmann (1980) has discussed the advantages and limitations of this technique. Again, the primary disadvantage of DCCC is the cost of instrumentation (ca. $10,000).

Identification of isolated and purified plant constitutents generally involves measuring a number properties, such as: melting point, boiling point, optical rotation, Rf or RRt values, and spectral properties, including ultraviolet-visible (UV-VIS), infra-red (IR), nuclear magnetic resonance (NMR, including C-13 NMR), and mass spectral (MS) measurements. Generally, UV-VIS and IR are routinely used in the identification of phytochemicals. Recent technological advances in microchips have made rather sophisticated UV-VIS spectrophotometers available at a very reasonable cost (e.g., $5,000-10,000). On the other hand, NMR and MS methods require considerable technical expertise and expensive instrumentation (e.g., typical NMR = ca. $60,000-100,000; typical MS or GC-MS = over $200,000; both NMR and MS require a full-time technician to operate and maintain the instruments). However, confirmed identification of each constituent is essential if precise characterization and evaluation of plant germplasm is to be achieved.

From a taxonomic/systematic standpoint, flavonoids have been the most widely used of all secondary constituents of plants (Harborne and Turner, 1984). This is due in large part to their ubiquitous occurrence in higher plants and their great structural diversity. In addition, they are relatively easy to isolate and characterize (Mabry et al. 1970). Flavonoids have been utilized as taxonomic markers at almost every level of systematic study, involving both wild and cultivated taxa. Extensive reviews of this area have been provided by several authors (Alston and Turner, 1963; Erdtman, 1963; Alston, 1967; Swain, 1975; Harborne, 1975; Harborne et al., 1975; Harborne and Mabry, 1982). In addition, flavonoids, as well as terpenes, have been used extensively in the chemical documentation of interspecific hybridization in plants (see Harborne and Turner, 1984 for a review of these data).

Phytochemical screening of plant germplasm, for the purposes of characterization and evaluation, has been employed by a variety of individuals with wide-ranging interests. Phytochemicals increasingly are becoming important sources of industrial and medicinal products (Balandrin et al., 1985). For example, compounds such as nicotine, the pyrethrins, and rotenone often are used as pesticides (Jacobson, 1982), and some steroids, alkaloids, and lignans are used by the pharmaceutical industry in drug manufacturing (Tyler et al., 1981). The flavors and aromas of many spieces, condiments and beverages (e.g., teas, coffee, colas) are, in fact, the result of the secondary products produced by the plants from which the products are obtained. Another area of potential agricultural significance is that of plant growth regulators obtained from natural plant sources (e.g., the steroidal lactone brassinolide obtained from the rape (*Brassica napus*; see Balandrin et al., 1985 and references therein). There is also considerable interest in the use of phytochemicals as supplements and/or replacements to petrochemicals (Wang and Huffman, 1981; Campbell, 1984). Of course, chemical characterization and evaluation of plant germplasm will continue to be of prime importance to plant breeders and systematists as they breed, select, evaluate, and attempt to understand the genetic and phylogenetic relationships of plants with existing and/or potential agricultural-industrial significance. For example, analysis of the flavonoids of pears and apples has provided useful data to support the recognition of these two important agronomic groups as distinct genera (*Pyrus* and *Malus*; Challice, 1973, 1974).

In summary, with respect to phytochemical screening, PC and TLC probably can be regarded as the "conventional" methods of analysis. These techniques have the advantage of being relatively fast and inexpensive. However, by themselves, they cannot provide data on the exact identity of specific chemical constituents. Instead, they can only confirm the presence or absence of compounds that have been identified utilizing the methodologies discussed above. These same criticisms can also be leveled against the more technologically advanced HPLC methods. Thus, it is safe to say that these newer technologies will not replace more "conventional" methods of phytochemical screening. Instead, they must be integrated with existing procedures to assure fast and efficient methods of phytochemical screening.

3. ELECTROPHORETIC TECHNIQUES

The concept of separating biological components by zone electrophoresis was described early this century (Field and Teague, 1907), and electrophoresis of amino acids on paper was a routine procedure in the 1950's. A major advance in the separation of proteins by eletrophoresis was the use of horizontal starch gels and a gel buffer of low ionic strength (Smithies, 1955). The use of activity stains for the visualization of specific enzymes was introduced by Hunter and Markert (1957). Electrophoresis on polyacrylamide matrix was introduced by Raymond and Weinstraub (1959) and this technique has been especially popular in extensions of the original discontinuous electrophoresis techniques (Kendell, 1928, 1942). Further advances in the separation of proteins have been made by using denaturing gels (Laemmli, 1970), isoelectric focusing (Wrigley, 1968) and 2-dimensional electrophoresis (O'Farrel, 1975). Finally, the electrophoretic techniques are amenable to and, indeed, are the primary methods used in separation of DNA fragments (Maniatus et al., 1982).

With respect to germplasm evaluations all electrophoretic techniques permit the assessment of genetic diversity at the molecular level but can provide little information on physiological or morphological diversity. Thus, such techniques can generate additional information but cannot supplant the data gathered by observations of growing plants. Each of the electrophoretic techniques mentioned above have been used for the evaluation of genetic diversity, and each also possesses certain advantages and disadvantages for such work. In order to better understand their different features each technique will be described briefly.

Horizontal starch gel electrophoresis: The use of this technique is almost always associated with isozyme analysis and has become the primary tool of assessing allozyme variation in populations (Brown, 1978, 1979; Jain and Singh, 1979; Kahler, 1981). Plant samples to be analyzed are crushed in a small volume (0.5 - 2 ml) of extraction buffer, the extract absorbed onto a filter paper "wick", and the wick inserted directly into a slot on the gel. Twenty to forty samples can be analyzed per gel, and because a relatively thick (1 cm) gel is used, this gel may be sliced, parallel to its horizontal surface, into 4-8 duplicate, but much thinner, gels. Each of the thin gels can be stained with a different assay, permitting each sample to be analyzed for 4-8 isozyme systems. On the average there are two loci expressed per isozyme system analyzed in plants (Gottlieb, 1982), so that 8-16 bits of genetic information can be obtained on one sample and 250-500 such bits can be amassed on the samples run on a single gel. An experienced technician can run and analyze 10 or more gels per day, and it is this ability to generate considerable amounts of genetic data that has made horizontal starch gel electrophoresis a particularly useful tool for the population geneticist.

Although the use of starch as a matrix gave clearer enzyme bands than paper and other media used in 1940 - 1950's, biochemists and other researchers requiring even better resolution soon shifted to a polyacrylamide matrix, for such a gel can be poured more uniformly and provide greater flexibility in the sieving properties, buffer and stacking systems, and assay compatibility. The concentration of the acrylamide subunit can be adjusted from around four to about 20% of the total gel. The mobility of high molecular weight molecules, such as proteins, is greatly affected by the gel concentration; the higher the concentration, the more retarded is the movement of the macromolecules through the gel. Many polyacrylamide systems include a "stacking" gel in which the sample is concentrated into a very thin band before entering the resolving gel (Chrambach, 1980). Gradient gels, which have a lower acrylamide concentration at the top (where the sample enters the gel) and gradually increases to a high acrylamide concentration at the bottom, afford even better resolution.

Special assays may be used on polacrylamide gels to assay for enzymes such as ribonuclease and amylase which, because of the opacity of starch, are not compatible with starch gels. When compared to starch, the disadvantages of polyacrylamide include the necessity of working with a toxic monomer when preparing the gel, fewer samples assayed per gel, and a significant increase in expense and technical expertise required. Polyacrylamide gels cannot be as easily sliced as starch gels and, thus, only one isozyme system is assayed per gel. Also, more care must be taken in the preparation of the samples.

A possible alternative to electrophoretic separation of proteins based on charge and mass has been to denature the proteins using sodium dodecyl sulfate (SDS) or urea and separate the monomers on SDS- or urea-containing polyacrylamide gels. The migration of SDS-denatured proteins is determined almost entirely by the size of the protein.

Isoelectric focusing, usually performed on polyacrylamide or agarose gels, provides the possibility of even greater resolving power. Initial attempts to separate proteins gave poorer resolution than simple electrophoresis (Tiselus, 1941). However, great improvements in resolving power came about when the technique was applied in a gel matrix (Wrigley, 1968). Further improvements in techniques and equipment have made isoelectric focusing a powerful method for separating proteins.

Two dimensional electrophoresis analyzes one sample using two types of electrophoretic techniques. The most common combination is isoelectric focusing in the first direction, followed by denaturation of the proteins and electrophoresis in SDS in the second direction (O'Farrel, 1975). This combination appears to give the greatest resolutions and allows researchers to separate complex protein mixtures such as are found in seed or leaf extracts (Brown and Flavell, 1981). Recently, Cremer and Van de Walle (1985) have demonstrated that over 500 moieties can be distinguished in extracts from green plant tissue, and even more proteins can be resolved in certain other tissues. Bahrman et al. (1985) have applied this technique in genetic studies of Douglas fir, a significant accomplishment considering the relatively high levels of phenolics and other interfering compounds in tissues from this species. Two major problems with this technique are (1) reproducibility of conditions of electrophoresis among (and sometimes within) labs and (2) the limitation of analyzing only one sample per gel.

In spite of the very different macromolecule being analyzed, electrophoresis of DNA is similar and uses much of the equipment as protein work. Electrophoretic analysis of restriction fragments is very similar to electrophoresis of protein on denaturing gels, for the components are separated on differences in size, with smaller fragments migrating further. High molecular weight plant DNA is isolated from a convenient tissue, such as young leaves, and treated with a restriction endonuclease (Beckmann and Soller, 1983). Electrophoresis of the resulting fragments can be performed either on agarose or polyacrylamide gels, the former being the matrix usually employed. The location of a band or bands homologous with a specific sequence of DNA can be visualized by a number of methods (Feinberg and Vogelstein, 1983; Leary et al., 1983; Murasugi and Wallace, 1984; Renz and Kurz, 1984) but the most common is the use of autoradiography requiring a ^{32}P label probe (Maniatus et al., 1982). At present such a method has been the only one successfully used on single copy sequences in plants (Polans et al., 1985).

Which electrophoretic technique a researcher chooses obviously depends on the type of results desired. The evaluation of genetic diversity mandates that a large number of samples be screened. Such a requirement indicates that two-dimensional gel electrophoresis will not be very useful for general surveys. The relatively expensive materials and significant technical expertise required for this technique further reduces its future potential as a practical technology for germplasm screening.

Electrophoresis on denaturing gels also appears to have limited potential for assessing genetic diversity. Not only does this technique require a rather complicated sample preparation but also the amount of genetic variability exposed is usually low. In peas, variation in seed proteins was examined by urea-PAGE (a denaturing system) and isoelectric focusing (Przybylska et al. 1983). Considerably more variation was revealed by the isoelectric focusing approach. Similarly, only three different phenotypes could be observed in the denatured seed protein pattern of 100 bean (Phaseolus vulgaris) lines (Brown et al., 1982), whereas isozyme analysis of approximately the same set of lines permitted the unique identification of over half the lines and the splitting of the remaining cultivars into small groups with identical isozyme phenotypes (Weeden, 1983a). The explanation for this low level of variability present on denaturing systems appears to be that proteins are more apt to vary in charge, which could be caused by a single amino acid change, than in subunit size.

Of the remaining three electrophoretic techniques, restriction digests seems to offer the greatest potential, for high levels of polymorphism have been consistently observed in barley (Saghai-Maroof et al., 1984), tomato (Tanksley, 1985), and pea (Polans, et al. 1985). One also has the possibility of working with any of three genomes (nuclear, plastid and mitochondrial), each of which may provide different information on the evolution and systematics of the group being investigated. However, this technique is somewhat difficult and expensive. Thus, its use would appear to be limited to major laboratories equipped with the apparatus necessary for work with radioisotopes and restriction endonucleases. At present this technique is being used by molecular geneticists primarily to study the structure and arrangement of genes (Saghai-Maroof et al., 1984; Polans et al., 1985) and by evolutionists and systematists to study the relationship and differences between taxa (Palmer and Thompson, 1981; Sytsma and Schaal, 1985). Characterization of germplasm represents only a secondary result of these studies.

The two techniques most frequently used to access genetic diversity have been (1) isozyme analysis on either starch or polyacrylamide gels and (2) isoelectric focusing. Of these, horizontal starch gel electrophoresis has probably been the most popular technique and has generated the majority of data. Major surveys of isozyme polymorphism have been performed in maize (Goodman, et al 1982); wheat (Hart, 1983); tomato (Rick & Forbes, 1975); pea (Weeden, 1983b; Alconero et al., 1985); and barley (Brown & Mundy, 1982). The most completely characterized species is probably maize (Zea mays L.) in which nearly all inbred lines and many hybrids have been characterized for allozymic genotype at 22 loci (Stuber and Goodman, 1983). In addition, limited surveys have been done on hundreds of other cultivars and wild species. Accurate labeling of samples by isozyme fingerprints is another application of this technique which may be especially important at seed distribution centers. A recently completed survey of the isozymes in selected pea accessions at the Northeast Regional Plant Introduction Station revealed instances of contamination and genetic drift which otherwise may have gone unnoticed (Alconero et al., 1985). The cost of equipment and supplies for horizontal starch gel electrophoresis is low compared to polyacrylamide gel electrophoresis (PAGE). The combination of low cost with the considerable amount of data that can be obtained from one gel accounts for the popularity of starch gels among researchers who measure genetic variation in large populations.

Isoelectric focusing usually gives better resolution than PAGE but requires more expensive materials and equipment. Superb studies on peroxidases and esterases in several taxa have been accomplished using isoelectric focusing (Hicks et al., 1982; Villamil et al., 1982); however, many enzymes are modified or denatured at their isoelectric point, interfering with their analysis by this method. The high cost of ampholytes and constant power transformers needed for isoelectric focusing together with the occasional generation of artifactual bands has hindered large scale use of this technique for germplasm surveys.

To summarize this comparison of electrophoretic techniques, it appears that isozyme analysis on starch gels, although one of the first methods developed, remains the most efficient and cost effective technique for assessing genetic diversity. PAGE and isoelectric focusing usually give slightly better resolution, thereby exposing more polymorphism but generate only about one fourth of the data per hour of technician time. Two-dimensional gel electrophoresis can isolate more gene products than single dimension techniques but is severely limited in the number of samples it can process, and the level of polymorphism revealed appears to be comparable to that for starch gels. The most recent method developed, DNA restriction fragment analysis, presently requires a procedure which is too sophisticated and expensive to be applied to general screening at germplasm facilities. However, it reveals considerably more polymorphism than the other methods and eventually may provide the best method for assessing genetic diversity.

The technical methodologies available for investigating botanical, agronomic, phytochemical, and electrophoretic variability discussed in this paper would greatly extend but not replace the 'conventional' analyses generally performed at Plant Introduction Stations. The weakness of the technological approach is not the lack of appropriate technology but a lack of knowledge regarding which traits should be analysed. Controlled environments permit the analysis of individual factors which normally cannot be studied in the field because of the compounding effects of many other variables. Such studies have already provided considerable information on which plant characteristics are important for meeting certain goals (Mann et al., 1985; Rajashekar et al., 1983). Further progress using more sophisticated techniques should permit a more precise understanding of these characteristics.

The evaluation of genetic diversity may be immediately extended by isozyme, protein, or restriction fragment length analysis. These approaches focus on DNA or gene products and provide a direct measure of genetic diversity often difficult to obtain by conventional methods. Analysis of phytochemicals provides a second, more indirect and probably less efficient method of measuring genetic diversity. Results from phytochemical or electrophoretic surveys often complement the morphological data, exposing variation in a morphologically homogeneous set of accessions or uniformity in an otherwise heteromorphic group. The disadvantage of these molecular techniques is that the variation exposed often has little direct value in terms of agriculturally important characters.

STRATEGIES FOR TECHNOLOGICAL DEVELOPMENT

Currently one of the major problems in germplasm evaluation is deciding which traits to evaluate. There exists a virtually unlimited number of morphological, physiological, and biochemical characters which could be studied, and a survey of 100 scientists would undoubtedly produce 100 different priority lists. In contrast, the facilities and funds available for evaluation purposes are quite restricted and woefully inadequate to thoroughly characterize all the accessions already catalogued into the Plant Introduction system. With more collections being made each year it is obvious that a very selective choice must be made regarding the traits to be evaluated. Factors that have to be considered include not only horticultural demands but also timelyness and efficiency. At present, high priority must be given to a more extensive characterization of most accessions so that (1) accessions may be reliably fingerprinted for purposes of quality control, (2) sources of new genetic variation may be identified and examined more closely, and (3) a better estimation of the total genetic diversity available in collections compared to that present in nature may be made. Two other areas also deserve emphasis with regard to germplasm evaluation. Basic research in plant physiology and biochemistry will enrich our understanding of the processes involved in specific plant responses to environmental stimuli. Such information facilitates the identification of easily tested plant characters that correlate with or will predict plant responses in the field. Screening for such characters during the primary evaluation of accessions would provide important data on the possible attributes of each accession. Finally, the evaluation of germplasm for specific traits crucial for current needs deserves special attention and support, for the use of the germplasm collection is one of the major goals of the sampling, storage and evaluation process.

The first area is especially important to note because it has been given the least support of the three even though it is essential to meet the goals of the germplasm repositories. Both basic and applied areas have been recognized for years as important aspects of agricultural research, and applied research, in particular, has received considerable support from both private and government sources. Fortunately, recent advances in technology provide greatly improved methods for characterization of the genetic variation present in accessions. For self-pollinating or clonal species displaying significant isozyme polymorphism (soybean, pea, inbred lines of maize) electrophoretic analysis on starch gels offers an inexpensive yet reliable method for characterizing accessions. Such analysis evaluates the genetic diversity within accessions, provides a measure of the genetic diversity between accessions, and differentiates among accessions using genetically defined polymorphism. Outcrossing species present some difficulties because many genotypes exist within each accession, and the genetic description given by the isozyme analysis is ony as good as the sample size. However, such data would often be the only information available on variation at the specific loci. Isozyme analysis can and should be a part of the characterization procedure performed on each accession of at least most species at the Regional Plant Introduction Stations. The primary obstacle to implementing such a program appears to be associated with the personnel rather than the technique, most of the employees at the stations being either administrative or field-oriented.

The isozyme data can be augmented and, perhaps, eventually replaced by techniques exposing the variation present in DNA sequences. However, several problems need to be resolved before restriction length fragment analysis can be conveniently used in general surveys. These include:

1. Establishment of a reliable protocol for DNA extraction.
2. Production of a blotting matrix which will not release bound DNA during washes.
3. General availability of plant genomic libraries.
4. Development of an alternative to radioactive labeling of probes for visualization of single copy DNA.

In order to perform restriction fragment length analysis one must be able to extract high molecular weight DNA from seed or leaf tissue. Several excellent procedures for the isolation of DNA from plants have been published (Appels and Dvorak, 1982; Saghai-Maroof, et al., 1984), but these procedures do not work for all species of plants. Before restriction fragment length analysis can be widely applied in the evaluation of germplasm a simple procedure, or limited set of alternative procedures, for the isolation of high molecular weight DNA must be established.

Much of the time required in DNA analysis involves the extraction , restriction, electrophoretic separation and blotting of the DNA. The reuse of a Southern blot, once made, would obviously increase the efficiency of the technique. Currently blotting material exists that can be reused several times, but what is needed to make the technique efficient enough for germplasm surveys is a material which can be blotted 10 to 20 times before being discarded. Such an advance would permit a reasonable characterization of an accession from only one or two blots, providing that enough different probes were available to generate 20 or 30 patterns.

This problem of probe diversity will ultimately be solved when DNA libraries are available for at least one species from the majority of plant genera. Many DNA segments are highly conserved and thus the sequence from one plant can be used to search for homologous sequences in widely divergent genomes (Hattori and Johnson, 1985). Many sequences, however, will hybridize strongly only with DNA from closely related species, thus different DNA probes are required for each genus being investigated. A sufficient diversity of probes is available only for a few of the major crops and a few species particularly useful for molecular genetic studies.

The final obstacle is a technical one that may be on the verge of being overcome. Although ^{32}P-labelled probes provide the only demonstrated means of visualizing single copy sequences in genomic blots of eukaryotic DNA, there are several techniques which may be able to identify single copy DNA under ideal conditions and can definitely be used for repetitive DNA sequences (Leary et al., 1983; Renz and Kurz, 1984). At present, however, this problem appears to be the most crucial of the four, for routine screening with radioactive probes would not be practicable in most germplasm facilities.

Other technological advances such as the use of computers to facilitate the collection and analysis of data, improvements in chromatographic and spectroscopic techniques for screening secondary products, adaptations in the use and design of controlled environmental chambers, immunological procedures and scanning electron microscopy will also find applications in the characterization of germplasm, although the extent to which these methodologies will be used still remains uncertain. A major constraint for the widespread application of many of these techniques, particularly in a university setting, is the cost of acquiring the necessary instrumentation. It appears, however, that this constraint will lessen with time as more cost efficient methods are employed in the manufacturing of these instruments (e.g., consider the drastic reduction in equipment costs in the computer and data processing fields).

Applications of new technologies to the basic research described earlier is limited only by the imagination of the scientist. Much of this research will be at the molecular level, attempting to understand why and how a plant responds to a particular stimulus. Thus, this research will rely heavily on technologies developed for the fields of biochemistry, molecular genetics, and physiology. In contrast, the third high priority area, that of meeting today's needs in horticulture, neither requires nor can use much of the technology currently available. In general, this situation arises because breeders are assessing traits such as seedling vigor, drought tolerance, and yield which are more reliably evaluated by direct observation or well established procedures than by any of the more advanced technologies.

In summary, there exists available technology which could make significant improvements in the evaluation of genetic diversity, in quality control practices, and in the generation of new information on how plants interact with themselves and the environment. Most of this technology is available at research institutions with close ties to the Plant Introduction program. It should, therefore, be relatively easy to incorporate much of the available technology into the germplasm evaluation process by increasing the interaction between the primary screening centers and the research laboratories. The information produced in all laboratories must be reported back to the primary centers which then summarize and cataloge all data on each accession.

There is an immediate need for an increased emphasis on the characterization and evaluation of the accessions currently held at Regional Plant Introduction Stations. Not only is such a step necessary before breeders can efficiently use the collections but a thorough characterization of the genetic diversity would undoubtedly reveal certain regions of a species' range displaying particularly high levels of genetic variability. Such areas should targeted for further sampling before the spread of civilization eliminates the native flora in these areas. This loss of germplasm is occurring particularly rapidly in the tropics. Most tropical plant species have not even been described, let alone evaluated for their chemical constituents or studied in a taxonomic/systematic sense. At current rates of destruction, plant biologists may have only 10-20 years in which to characterize and evaluate much of the world's flora for potential sources of agricultural, industrial, and medicinal chemicals. Conservation of this biological diversity must become high priorities of the agricultural and scientific communities if we are to have the natural resources available to which these emerging technologies can be applied.

INSTITUTIONAL ACTIONS

Most of the objectives discussed in the previous sections (application of currently available technology to germplasm evaluation, stricter demands for feedback of data for evaluation studies, and support for basic and applied research) can be attacked at the institutional level. Only the further development of methodologies, specifically those involved in restriction fragment analysis, appears to depend on the talents of individual researchers in the field more than any program that could be initiated by larger organizations.

Of the high priority areas mentioned, the one requiring the most help immediately is the primary characterization and evaluation of germplasm as carried out at Regional Plant Introduction Stations and other designated crop research centers. At present, these facilities are an under-utilized resource, for technologies now exist appropriate for implementation at such centers which would greatly increase the reliability, efficiency and utility of the screening process. Current characterization practices are often inadequate because the traits chosen fail to clearly differentiate among accessions, only vaguely describe the genetic diversity of the sample, and often provide breeders with little useful information. Fingerprinting by isozyme or DNA techniques would establish a reliable genetic description for each accession. Although the fingerprint, in itself, would not be useful to breeders, it would provide information on how much variation existed within the accession and how divergent the accession was from breeding stocks already sampled by the breeder. Similarly, methods for the analysis of certain phytochemicals in various taxa have been established and can be used to augment the genetic diversity evaluation in many taxa without major commitments of personnel or money.

It is important that such screening become incorporated into the primary screening process as soon as possible to give field botanists better information concerning the genetic variability within their collections and perhaps reveal geographical areas of high or unique diversity. Such areas could then become the focus of collecting expeditions before the habitat is significantly altered by the activities of man.

Greater interaction of the P.I. Stations with crop advisory committees, universities, and research centers would help establish improved descriptor lists and greater opportunities to evaluate for characters requiring equipment or expertise not available at the stations. It should also become mandatory for researchers requesting seed from P. I. Stations to submit a report of their findings. Thus, the dissemination of information on these accessions would become centralized and presumably more efficient. Such an arrangement would also minimize the number of repetitive or overlapping studies being done on the same samples.

Basic research in plants is currently being adequately funded by the U.S. Department of Agriculture, National Science Foundation, and several private institutions. Although most of this support is not directly involved with research on germplasm evaluation, many of the projects will provide information useful to the evaluation process. However most of these projects are being performed in laboratories that have little interaction with personnel involved in germplasm evaluation. Thus, a mechanism for the transfer of information from basic research to germplasm facilities would be highly desirable. Perhaps the most convenient solution would be to expand crop advisory committee memberships to include at least one molecular biologist working on the crop.

Federal and state funding for applied research is decreasing for most crops, and it is questionable whether private industry will ever match the support previously enjoyed by breeders. The current excitement about the possibilities of transferring specific genes into plants using recombinant DNA technology further undermines the attention given to conventional breeding bractices. Yet, at present, the needs of agriculture cannot be met by the new biotechnology, and the tedious but reliable process of identifying practical characters in exotic lines, and moving the genes responsible for such characters into a genetic background more suitable for cultivation must be supported.

SYNOPSIS OF RECOMMENDATIONS

(1) Initiate a 10 year program to complete a detailed evaluation of the genetic diversity and potentially useful agronomic characters in cultivated species and their relatives. The program should be centered around the Regional Plant Introductin Stations and other germplasm research centers and should have a working budget of $5 million per year. The program would have specific goals similar to the following:

(a) Provide funding for equipment and personnel to obtain genetic fingerprints of most accessions, using either isozyme or DNA polymorphism.

(b) Increase collaboration between Station personnel and scientists outside the Plant Introduction system in order to expand the expertise available for evaluation germplasm.

(c) Generate a detailed file on each accession by requiring users of the lines to report back on their findings, and summarizing this information in a computer accessible format.

(d) Encourage greater involvement of the crop advisory committees and breeders in determining which plant characters should be evaluated.

(2) Maintain funding for basic plant research at the current level while encouraging interaction between scientists performing such research and personnel in the plant introduction program.

(3) Encourage greater use of germplasm collections by breeders and other plant scientists through a special small grant program with a funding limit of $10,000 per year per grant.

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APPENDIX I

PEA DESCRIPTORS

Column No.

1 Plant Introduction Number

2 Origin

3 *Plant Height at Maturity*. Plant height in cm from ground to tip at maturity of a typical plant.

4 *Plant Height to First Flowering Node*. Height in cm from ground to first flowering node of a typical plant.

5 *Node Number to First Flower*. A count from the second trifid bract as one, up to the first flowering node when 50% of the plants in a planting are flowering - expressed as an average of five randomly chosen plants (in integers).

6 Leaflet Number at 1st Flowering Node. Counted on a typical sample.

7 *Leaflet Morphology*

1. Afila
2. Normal
3. Acacia
4. Other
5. Mixed

8 *Stipules*.

1. Normal
2. Reduced
3. Mixed

9 *Flower Color*.

1. White

2. Pigmented
3. Mixed

10 Stem Fasciation.

1. Yes
2. No
3. Mixed

11 Flower Number. Maximum number of flowers per peduncle.

12 Days to 50% First Flower. Number of days from day of planting to the day when 50% of the plants in a planting are at the first flowering stage (both days inclusive).

13 Heat Units to 50% First Flower. A summation of all the heat units from the day of planting to the day when 50% of the plants in a planting are at the first flowering stage. The heat units are calculated for every day as:

$$\frac{\text{Daily Maximum Temperature} + \text{Daily Minimum Temperature}}{2} - 40^{\circ}\text{F} = \text{Heat Units}$$

14 Pods Per Peduncle. Counted as an average of three peduncles per plant from five typical plants of a planting - expressed up to first decimal place.

15 Number of Ovules Per Pod. Average number of ovules from five longest pods, each from five different plants at immature pod stage.

16 Pod Shape. Measured at flat pod stage.

1. Straight
2. Curved

3. Mixed

17 _Pod Apex_. Measured at flat pod stage.

1. Blunt
2. Pointed

18 _Pod Type_.

1. Non-edible
2. Edible

19 _Pod Length in cm_. Average length of five largest pods, measured from five different plants at full pod stage, expressed with one decimal place.

20 _Pod Width in cm_. Average width of five largest pods (each from a different plant), measured between sutures at full pod stage, expressed to one decimal place.

21 _Pod Color_.

1. Green
2. Other

22 _Number of Seeds Per Pod_. Average number of seeds from five longest pods, each from five different plants at maturity.

23 _Seedcoat Color_.

1. Pigmented
2. Non-pigmented
3. Mixed

24 _Hilum Color_

1. Clear
2. Pigmented
3. Mixed

25 *Cotyledon Color*. Evaluated from unweathered, unbleached pods.

1. Yellow
2. Green
3. Other
4. Mixed

26 *Seed Surface*.

1. Smooth
2. Dimpled
3. Wrinkled
4. Mixed

27 *Weight of 100 Seeds*. Weight of 100 seeds in grams measured at seed moisture equal to or less than 12%.

28 *Virus Rating*.

1. Slight infection
2. Heavy Infection

29 *Water Congestion*

1. Present
2. Absent

30 *Planting Date*. Month, day and year on which the accession was planted for purposes of evaluation.

Contract Paper No. 7

Assessment of Plant Quarantine Practices

Robert P. Kahn
USDA-APHIS-PPQ

November 1985

Office of Technology Assessment
U.S. Congress

This contract paper was prepared for the assessment of "Technologies to Maintain Biological Diversity." The views expressed are those of the author(s) and not necessarily those of OTA.

TECHNOLOGIES TO MAINTAIN BIOLOGICAL DIVERSITY: ASSESSMENT OF PLANT QUARANTINE PRACTICES

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DISCLAIMER

This document is not meant to serve as a source of information about the entry status of imported articles in general, or plant germplasm in particular. Rules of and regulations of the United States and other countries are subject to change.

Persons desiring up-to-date information about the quarantine aspects of plant germplasm imports should contact the Permit Unit, Plant Protection and Quarantine (PPQ), Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture (USDA), Federal Building, Hyattsville, Maryland 20782.

Persons desiring information about the plant exports should contact the Export Certification Unit at the same address.

Information about imports and exports also can be obtained by contacting any PPQ, APHIS, USDA office listed under the U.S. Government in telephone directories.

ACRONYMNS

APHIS	Animal and Plant Health Inspection Service, USDA
ARS	Agricultural Research Service, USDA
CFR	Code of Federal Regulations; Title 7, Agriculture; Chapter III
CGIAR	Consultative Group on International Agricultural Research
CSRS	Cooperative State Research Service, USDA
FAO	Food and Agriculture Organization of the United Nations
FPPA	Federal Plant Pest Act
FNWA	Federal Noxious Weed Act
IPPC	International Plant Protection Convention of 1951
IR	Inter-Regional Projects, CSRS
PGQC	Plant Germplasm Quarantine Center, Beltsville, MD
PPQ	Plant Protection and Quarantine
PQA	Plant Quarantine Act
PQF	PPQ Plant Quarantine Facility, Beltsville and Glenn Dale, MD
USDA	U.S. Department of Agriculture

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1.0 INTRODUCTION

1.1 OBJECTIVES

The objective of this paper is to provide information, data, options, and recommendations concerning plant quarantine practices and their impact on the exchange of plant germplasm. As such, the paper supports the Office of Technology (OTA), U.S. Congress, assessment of "Technologies to Maintain Biological Diversity."

The report addresses the following topics: (1) Plant quarantine and biological diversity background information, (2) exotic pest threats and natural and man-made pathways, (3) germplasm in a pest risk/exotic pathogen perspective, (4) exclusion as a regulatory action, (5) existing and "new" pathogen detection procedures, (6) constraints on the flow of germplasm, and (7) options and recommendations to facilitate the safe, but timely transfer of plant germplasm.

1.2 SCOPE

The report covers the interaction of quarantine and the biological diversity developed from natural selection or classical genetics (Groups 1 and 2 below) rather than diversity obtained by recombinant nucleic acid genetic engineering (Group 3 below).

Group 1: Biological diversity as it may occur on the farm or in nature.

Group 2: Biological diversity developed through classical genetics such as conventional breeding between plants which exchange genetic material in nature or by the use of mutagens.

Group 3: Biological diversity developed by genetic manipulation of organisms through recombinant nucleic acid engineering or other techniques that enable the exchange of genetic material between species or genera which do not do so in nature or in conventional breeding.

This report covers sources of biological diversity in Groups 1 and 2 as they may be imported into or exported from the United States including its

Territories, Districts, and Possesions. Plant materials and biological diversity described in Group 3 are beyond the scope of this report since Federal positions, policy, and rules have not been developed, although a preliminary policy statement has been published in the Federal Register dated December 31, 1984. The importation of plants in Group 3 would not only be subject to restrictions based on quarantine rules but may be subject to rules developed in the United States and other countries to meet risks associated with genetic manipulation.

The terms "plant pest" or "pest" as used in this review are defined in Federal statutes and regulations (USDA, 1984). A plant pest is "any living stage of any insects, mites, nematodes, slugs, snails, protozoa, or other invertebrate animals; bacteria, fungi, other related plants or reproductive parts thereof, viruses, or any other organisms allied with any of the foregoing, or any infectious substances which can directly or indirectly injure or cause plant disease." Noxious weeds as listed in the FNWA may be added to list of pests. The term "pest" in foreign regulations may also include all weeds, rodents, bats which feed on plants, and predatory birds. Within the scientific community, it is customary to divide these pest groups into pathogens (fungi, viruses, bacteria, virus-like organisms) and pests (all others), with nematodes considered by some specialists to be pests and by others to be pathogens. However, in regulatory circles and in this report "pest" refers to all injurious or pathogenic organisms that damage plants.

1.3 BIOLOGICAL DIVERSITY

Biological diversity is a characteristic of living organisms (including viruses or virus-like entities) and reflects genetic variability which determines how well the species individuals or populations will survive. A species is a man-made taxon representing the lowest level in a taxonomic hierarchy that has been attached to individuals sharing certain defined characteristics. Nevertheless, variation occurs within the species levels. Groups or sub-groups within the species reflecting this variation are called strains, cultivars, pathovars, varieties, breeding lines, isolates, races, forms, biotypes, land races, etc. In the short term, in nature, it is these variations that ensure that individuals of the species will complete a life cycle or generation; and in the long term, that the species will survive in an evolutionary sense. In crop improvement, variation that exists among individuals is identified, collected, conserved, and utilized not only to ensure survival of the wild species and land races but to improve or diversify agriculture.

Biological diversity includes the variation in all organisms, including crop and pest species. Among plant explorers and breeders, biological diversity connotes the benefits to be accrued by the collection, conservation, and utilization of genes that contribute to the variation in wild and cultivated plants throughout the world. The assembled genes are channeled to develop new varieties, increase crop production, increase adaptability to environmental conditions, increase pest resistance, alleviate famine, or improve human and animal nutrition. Among pest control specialists and quarantine officers, biological diversity also connotes the possibility of the inadvertent, but simultaneous collection of detrimental phenotypes. Plant species have been introduced but have escaped and become troublesome weeds; e.g., kudzu vine, johnsongrass, multiflora rose. Similarly, insects introduced for beneficial purposes (MacGregor, 1973); e.g., gypsy moth, Africanized bee in South America, have escaped and become pests.

The objective of a biological diversity program operating under quarantine procedures and restrictions is to filter out harmful or hazardous species (quarantine) while channeling in beneficial species (plant introduction). While the filtering out of exotic pest individuals and species can be accomplished by inspection and treatment upon entry for many pest/host importations, the process for other interactions is more complex. For these cases, such filtering requires the detention of imported plants while tests for the detection of obscure pathogens (mostly virus, virus-like organisms, bacteria, and obscure fungi) are conducted. Unfortunately, the release of the importation may be delayed by the need to conduct tests. Testing requires not only personnel, well-equipped laboratories and greenhouses, but time. It is this time constraint, 2 to 5 years particularly for the detection of certain viruses and virus-like organisms in woody plants, which impacts on germplasm importation programs.

1.4 PLANT QUARANTINE

1.4.1 PRINCIPLES

Plant quarantines, rules, actions, or procedures are established by a government or a group of governments to prevent the entry of pests of quarantine importance in or on articles imported along man-made pathways as discussed in Section 2.4 of this report. Among regulated articles are plants, plant products, soil, cultures of living organisms, packing materials, commodities, nonagricultural cargo, and used vehicles and farm equipment as well as their containers and means of convenience.

Among the concepts used by governments in promulgating rules or taking regulatory actions are the following:

1. Quarantines should be biologically based and not used as trade barriers or for other political and economic reasons.

2. Actions taken should be the least drastic action commensurate with the known or perceived level of risk associated with the article to be imported (e.g., plants, seeds, cuttings, tubers, budwood, etc.). The least drastic action is the least restrictive action that will effectively exclude the pest. (Foster 1981, Kahn 1982a).

3. Benefit/costs and benefit/risk considerations should be included in taking any regulatory position. Some risks may be taken on a benefit/risk analysis if the benefits are crop improvement and diversification.

Among the regulatory actions that can be taken against imported articles in general, or plants or plant parts in particular, are the following arranged in the order of the most drastic action first:

1. Prohibit or exclude the plant material.

2. Allow entry of prohibited plant material (subject to inspection and treatment, if necessary, or denial of entry, depending on pest finds upon entry) and then require passage through a quarantine station or other special containment facility.

3. As in 2, but allow planting in isolation under postentry quarantine on the property of the importer.

4. Require a mandatory treatment at origin or upon entry whether or not a pest is found.

5. Require inspection as of condition of entry and if a pest of quarantine importance is found--

   (a) Destroy the consignment;

   (b) Deny entry and allow the consignment to be returned or reexported;

   (c) Treat the entire consignment, if an effective treatment is known;

   (d) Treat only the plants or plant parts which are infected or infested; or

   (e) Excise infected or infested portions of a plant or remove infected or infested plants and release the remainder.

1.4.2 LEGAL BASIS OF QUARANTINES

The legal basis for quarantine action in any country is either the legislation passed by National, and sometimes State or Provincial, governments as acts, orders, rules, or regulations, or enabling legislation that authorizes the Minister of Agriculture to issue rules, orders, or directives.

In the United States, the legal authority rests in statutes such as the FPPA, the PQA, and the FNWA under which rules or regulations are issued (U.S. Department of Agriculture, 1984).

The legal umbrella under which international plant quarantine activites are covered is the IPPC of 1951, also known as the "Rome Convention." The IPPC provided the international model for the phytosanitary certificate which accompanies certain articles in international movement. The IPPC is a treaty and the United States is a signatory nation. The phytosanitary certificate is an instrument of that treaty. Groups of governments such as the European Economic Community have a parliment that promulgates quarantine directive that are binding upon member countries.

1.5 PEST RISK ANALYSIS

The biological basis for promulgating regulation, rules, and regulatory actions is the perceived or known pest hazard and risk associated with the entry of an imported article including plants, seeds, cuttings, tubers, etc., (Kahn, 1979a). Pest-risk analysis is a thought process whereby the entry status of imported articles is determined based on the calculated risk of inadvertently introducing hazardous pests and pathogens on these items as moved by man.

"Entry status" refers to the entire range of decisions, policies, rules, or regulations that govern whether or not an article is enterable, and if enterable, under what safeguards. Attitudes toward entry status may range from "conservative" to "liberal," but "liberal," in this context does not imply "lax." The most liberal attitude is that plant propagative material may enter freely without agricultural regulatory restrictions. The most conservative attitude is that the material is excluded without any exceptions even for scientific purposes. United States rules do not prohibit without exception nor are plant propagative materials freely enterable without any restriction.

The pest risk analysis concept (Kahn 1979a), which provides the basis for the entry status determinations for germplasm, is presented in Appendix A and illustrated in Figure 1. The terms "hazard," "risk," "safeguard," and "benefit/risk considerations" are defined in Appendix A.

1.6 CHARACTERISTICS OF PLANT QUARANTINE REGULATIONS

Quarantine regulations and procedures in the United States and in most other countries of the world--particularly those that adhere to or are guided by the IPPC--have the following characteristics:

1. Specify prohibitions, exclusions, or other regulatory actions
2. Grant exceptions to prohibitions for scientific purposes to allow entry under safeguards
3. Require import permits
4. Require phytosanitary certificates
5. Stipulate inspection upon arrival
6. Prescribe treatment upon arrival to eliminate a risk
7. Prescribe quarantine, postentry quarantine, isolation, or other safeguards

Standards or guidelines for establishing regulations are often suggested by international plant protection organizations (e.g., EPPO, NAPPO, the Inter-African Phytosanitary Council, and other regional organizations for the Near East, Southwest Pacific, the Caribbean area, and South America).

1.7 INDEPENDENT SAFEGUARDS

A safeguard is an action taken to reduce the chances that an article in general, or a plant in particular, might be carrying or harboring a plant pest of quarantine significance. An independent safeguard is one that has a different mode of action than another safeguard. Thus, inspection and treatment are independent safeguards. Inspection at origin and inspection upon entry are two safeguards, but their modes of action are the same. Therefore, they are not independent safeguards. The greater the number of independent safeguards the lower the chance is of a pest entering on an imported but safeguarded article. The seven characteristics of quarantine regulations shown in 1.6 are safeguards.

2.0 THE THREAT OF EXOTIC PESTS

2.1 CENTERS OF ORIGIN OF CROPS AND PEST SPECIES

The centers of diversity or origins of a crop species have been considered to be the area, or areas, in which the largest number of genetic varieties occur. Such areas are now referred to as primary centers (areas where the crop species evolved) and secondary centers (areas where the crop species is grown elsewhere in the world) (Zohary, 1970; Kahn, 1977).

At the primary centers, in particular, or in the wilds, plant and pest species coexist in an interaction where each adjusts through genetic selection to the population of the other. Thus, in theory, the place to look for resistance in coffee to the coffee rust fungus is in areas where the two coexist--but being careful not to collect propagules of the rust fungus which in itself is adjusting to a more resistant coffee population by variations in its own pathogenicity and range.

The center of origin concept is particularly applicable to pests with narrow host ranges such as the coffee/coffee rust interactions. However, even pest species with wide host ranges may have subgroups with limited host ranges.

2.2 GEOGRAPHIC DISTRIBUTION OF PESTS

The present distribution of pest species represents the sum of the contribution to spread in the past by both man and nature as discussed in 2.4. Some pest species now are worldwide in distribution having the same ecological range as their hosts. However, many pest species are not found in the full ecological range of their hosts. The known distribution may be confined to a few countries or a continent. Even a pest species which has a wide distribution may have strains which are still of a limited distribution. Biogeographical concepts have been reviewed (Baker, 1975; Mathys, 1975; Kahn, 1977; Rapoport, 1976).

Propagules of pest species may arrive at a new location as a result of man´s activities or some feature of the pest life cycle that makes it readily disseminated by natural means. Pest establishment, after entry, which is beyond the scope of this report, depends on the presence of susceptible hosts, a favorable environment and sufficient inoculum or individuals of the pest species.

2.3 EXOTIC PLANT PESTS OF QUARANTINE SIGNIFICANCE TO THE UNITED STATES

United States quarantine rules and procedures are designed to ensure that imported articles are free of exotic pests of quarantine significance. No attempt is made to ensure that such articles are free of all pests and pathogens. Consequently, the definition of "quarantine significance" is important because rules and regulatory actions are instituted against quarantine significant pests.

An exotic pest is considered to be of quarantine significance if--

1. It is known to cause economic damage, or based on its life cycle, is capable of causing such damage; and

2. It is not known to occur in the United States; or, if it does occur, it is not widely distributed; or is under a Federal domestic quarantine program for eradication, containment or suppression; or represents a particularly virulent strain of a domestic pest; and

3. It cannot be inspected for by procedures routinely available at a Plant Inspection Station at a U.S. port of entry; or, there is no known effective eradicant quarantine treatment to apply if the pest is found as a result of inspection.

A list of pests mentioned by name in U.S. quarantine regulations is presented in Appendix B. However, this list is not meant to be all-inclusive. Quarantine rules in the United States and elsewhere, in listing exotic pests of quarantine significance, often use the phrase "including but not limited to the following pests." It is not biologically feasible to make a complete listing because pest/host interactions, agricultural practices, and geographic distribution of pests change over time. The threat to the agriculture of North America of exotic pests has been reviewed for plant diseases (Schoulteis, 1983), weeds (Zimdahl, 1983), and insects (Lattin, 1983). An estimate of the number of major exotic insects and disease agents which threaten the United States is presented in Table 1. An estimate of the number of exotic seed-borne pests is shown in Table 2.

2.4 PATHWAYS FOR THE ENTRY OF PLANT PESTS

Exotic pests can be moved along natural or man-made pathways (Figure 1 and Table 3) (Kahn, 1977; 1982a, 1982b, 1984). Whether a pest moves along a natural pathway depends primarily on the life cycle of the pest. Some species produce airborne or vector-borne propagules and are readily spread over long distances. Others may move by the cumulative effect of a series of short distance movements over a long period of time until a natural barrier (including the absence of a susceptible host) is reached. However, many of the viruses, virus-like pathogens, and bacteria without mobile vectors, most nematodes, many fungi without airborne spores, and some insects such as scales are seldom spread far naturally, although they may be moved over long distances by man along man-made pathways. Whether a pest moves along man-made pathways depends on the activities of the traveling, scientific, and commercial publics; the hitchhiking ability of some pests, and the effectiveness of quarantine safeguards. When nature does have an effective way of moving pests long distances, quarantine restrictions become less meaningful and less successful. Consequently, quarantine rules and restrictions are usually directed towards pests that are moved primarily by man rather than by nature.

3.0 PLANT GERMPLASM AS A PATHWAY

Plant pests of economic and/or quarantine significance can be moved far by natural means and also in, on, or along with articles moved by man. Of these articles, living plants or plant parts present the greatest hazard with as carriers or vectors of:

1. Obligate parasites that require living plant material to complete a life cycle;

2. Facultative parasites that require living plants at least during part of the life cycle; or

3. Other pests with no natural means of long distance spread.

When all the ways of moving plants and plant parts are considered (Table 3), the importation of plant germplasm has the greatest potential for facilitating the transfer of exotic pests. Much of the germplasm is collected in the wild, in markets, or in remote agricultural areas where the pests have not been identified. These collection sites may be in the center of origin or diversity for the host and its pests.

The risk is not significantly ameliorated if the collector is a scientist. Even if unhealthy-looking plants are avoided, latent or obscure pests are likely to be collected, particularly in vegetative propagules. Much of the plant material is collected leafless as seeds, fleshly underground storage organs, or dormant scions or plants. Consequently, neither observation by a collector nor inspection by a plant quarantine officer using facilities and equipment available at a port of entry would detect any latent or obscure pests.

3.1 IMPORTED GERMPLASM

Plant pests have been intercepted on imported plant material in general and germplasm in particular. The incidence of the detection of pests which require a regulatory action at U.S. ports of entry have been reported periodically (USDA, 1973-1984). For example, during the period between FY 1980 and 1984 (inclusive), 10,563 pests of quarantine importance were detected on restricted (i.e., subject to inspection upon entry) and postentry importations of plants or plant parts. These include 183 nematodes, 81 mites, 1,724 disease agents, and 8,627 insect interceptions.

Pests have also been detected on germplasm in quarantine stations in the United States. Prohibited plant material in quarantine at the USDA Plant Quarantine Facility at Glenn Dale, Maryland, and other locations have been tested for obscure pests such as viruses (Kahn 1967; 1970a; 1970b; 1979b). For example, between 1957 and 1967, 62 percent of the 1,277 introductions of _Citrus_, _Prunus_, _Malus_, _Pyrus_, _Solanum_, and _Vitis_ (Kahn 1967) were infected with viruses or virus-like pathogens. Between 1968 and 1978, 53 percent of 518 introductions of fruit trees (_Citrus_, _Cydonia_, _Olea_, _Prunus_, _Pyrus_, and _Malus_) were infected with one or more viruses or virus-like pathogens (Kahn 1979b) (Table 4). The incidence of the detection of virus in wild and cultivated germplasm is shown in

Table 5 for Solanum and in Table 6 for Arachis. Pests detected on plant material at U.S. ports of entry on plant material including germplasm are periodically tabulated and published (USDA, 1973-1984) germplasm collection sites.

Whatever the level of risk associated with the importation of a given species from a given country, the risk may vary with the collection site. Depending on where the plant or seeds were grown at the time of collection, the risk may vary. For example, the following collection sites are listed in the order of the highest risk first: (1) The wilds or the wilderness, (2) market places, (3) farms, (4) plant nurseries, (5) field plots or research stations (6) greenhouses, (7) tissue cultures, and (8) quarantine stations. The risk decreases as the ability to inspect and exclude pests increases. Similarly, the following collection sites are listed in the order of the highest risk first for introducing soil borne pests: (1) Wilds or wilderness, (2) cultivated rows, (3) ground beds, (4) potted plants on plastic sheeting, (5) raised benches outdoors, (6) greenhouse floor beds, (7) greenhouse raised benches, (8) plant growth rooms, and (9) tissue cultures. The risk decreases as the ability to use sterilized soil or no soil and to exclude soil or organisms increases.

3.2 EXPORTED GERMPLASM

Exported and imported germplasm move along the same pathways but in different directions. Consequently, any comments about imported germplasm as a pathway relate to exported germplasm. The principal difference is the quarantine significance of the pests which move on these pathways. Because of differences in the geographic distribution of pests and differences in crops grown, countries have differences in the pests they regulate against. Thus, the United States is concerned about exotic pests in Europe and Europe may be concerned about exotic pest in the United States—yet both may still be concerned about the exotic pests from South America (See section 2.2).

U.S. exporters of germplasm encounter problems in gaining acceptance of their materials. Quarantine officers tend to be conservative--particularly, when they do not have much information about foreign pest distribution. These export problems have been reviewed (Kahn 1979a, 1982b, 1983b, 1984b), and the topic discussed in Section 9.

3.3 INTERNATIONAL AGRICULTURAL RESEARCH CENTER

Germplasm is imported and exported by the International Agricultural Research Centers that form part of the CGIAR network. These centers, located in Mexico, Colombia, Peru, Nigeria, India, and the Philippines, export and import thousands of accessions of germplasm each year. The author reviewed (Kahn, 1982b by 1983; 1984) the plant health standards, procedures, and safeguards practiced by these centers in cooperation with various quarantine services. The review covered a total of 19 crops planted as vegetative propagations or seeds. Procedures, pathogen detection, phytosanitary standards, and criteria were discussed.

4.0 EXCLUSION AS A REGULATORY CONTROL MEASURE

Exclusion is the most drastic regulatory action that can be taken against hazardous exotic plant material. Exclusion or prohibition of the host is part and parcel of the regulations of almost all countries including the United States. However, exclusion is not an action taken frequently by governments (Kahn, 1982a, 1983b, 1984a). Most countries have exceptions that allow prohibited germplasm to enter under suitable safeguards.

The author conducted a survey of the regulations of 125 countries to determine to what extent plants were prohibited from one or more countries (Kahn, 1982a). Of the 245 different species listed, the 38 most frequently prohibited are shown in Table 7. These same regulations contain the names of 1,588 pest species or genera. The range among 125 countries of plant genera prohibited was 0 to 132 and the average was 15. The range of pests named in these regulations was 0 to 275 and the average was 355. The survey showed that there were at least 11 ways by which plants could be directly or indirectly excluded in regulations.

5.0 REGULATORY ASPECTS OF U.S. GERMPLASM IMPORTATIONS

5.1 REGULATIONS AND RESTRICTIONS

Plant material, in general, and germplasm in particular, may enter the United States in any of the following categories (Crooks, 1983; Foster, 1981; Rohwer, 1979):

1. Preclearance
2. Restricted
3. Postentry quarantine
4. Prohibited requiring a Departmental permit

5.1.1 PRECLEARANCE

Preclearance programs result from an agreement between a foreign plant quarantine service and PPQ under which certain low hazard plant materials are grown according to agreed upon safeguards. The foreign plant protection service inspects during the growing season, harvest, storage, packing, and shipping. The system is monitored by PPQ officers stationed in that country. Currently, the system is in place only for gloxinia, ferns, begonnia, African violets, peperomia from the Netherlands and certain perennial and ornamental bulbs from the Netherlands, Belgium, Israel, South Africa, and the United Kingdom. Scientists wishing germplasm produced under preclearance could import with no constraints. However, there is not much demand for germplasm available under this program.

5.1.2 RESTRICTED

Restricted is the category under which most germplasm imported into the United States enters with a minimum of constraints. Restricted means that a permit is required (usually written) and the material must enter through a PPQ, APHIS, Plant Inspection Station located at 1 of 14 ports of entry. Most vegetable, ornamental, foliage, and field crop seeds, as well as vegetative propagations of most plants enter as restricted. Once the material has cleared inspection, there are no other restrictions. The constraints are limited to the inconvenience of obtaining a permit, routing importations through inspection stations (as stated on the permit), the 1 to 3 day delay for inspection, and treatment, if necessary.

5.1.3 POSTENTRY QUARANTINE

Some germplasm enters under postentry quarantine. After plants pass through an inspection station they may be planted at an approved site under a growing agreement previously signed by the importer. Plants under postentry are examined periodically by a Federal and/or State inspector during detention period which is usually 2 years. If no exotic pests are detected, the material is released. Meanwhile, the importer may propagate the importation or use it for scientific purposes such as breeding. Constraints consist of the 2-year detention period; the need to have the growing sites approved in advance; and the requirements for isolation, labelling, and inspection. A problem exists in the 2-year detention period may not be long enough for the expression of symptoms for some agents.

5.1.4 DEPARTMENTAL PERMIT

Certain plants or plant parts used for propagation represent the greatest risk to U.S. agriculture because they may be infected or infested with exotic pests which cannot be detected by routine examination at ports of entry. These plants are prohibited to the general public but can be imported in small quantities by scientists under Departmental permit for scientific purposes. Safeguards are specified in each permit on a case-by-case basis. These safeguards usually involve growing the plants under the control of a knowledgeable specialist and subjecting the plants to certian tests designed to detect pests of quarantine significance and implementing phytosanitary procedures. Examples of plants in the prohibited group are shown in Table 9.

Departmental permits are issued to authorize the importation for scientific purposes of prohibited plants. Some prohibited material must pass through the PPQ plant quarantine facilities at Beltsville or Glenn Dale, Maryland. These plants include *Prunus*, *Pyrus*, *Malus*, *Solanum*, *Ipomoea*, several ornamental genera, grasses(collected as vegetative propagations) and cassava. Sugarcane passes through an ARS/PPQ quarantine in Beltsville or Glenn Dale, Maryland. *Vitis* may enter through several quarantine facilities at various locations; currently, entry is through California, Oregon, and New York. *Citrus* enters through Glenn Dale, Maryland, followed by transfer to continued quarantine in Gainesville, Florida, or Riverside, California. Other genera may pass through a containment in greenhouses or growth chambers under specified safeguards.

Plants which are in the prohibited category have the most constraints placed on them because the hazards and risks are the highest. The principal constraints are:

1. Imported material must be propagated and tested in high containment greenhouses with controlled temperatures to accommodate both temperate and subtropical plants.

2. Lack of space is often a limiting factor and could cause a backlog situation.

3. Obscure pathogen detection requires trained personnel and well-equipped laboratories.

4. Plants not requiring testing must be retained in quarantine at least two growing seasons which requires about 2 years of detention.

5. In addition, some plants must be detained until the completion of the pathogen detection tests which could take an additional 2 to 3 years for woody plants because viruses move slowly in woody plants.

6. Only small amounts of each accession may be imported since each propagative unit (to be used as a mother plant for furnishing material for release) must be tested.

7. Some accessions may arrive in poor horticultural condition and may not be established so that additional material may have to be requested increasing the detention period.

8. For vegetatively propagated germplasm, only progeny descended from tested mother plants is eligible for release. Consequently, space and time are required for propagating plant material for released to scientists.

9. Many plant species have never been studied for pathogens. Preliminary studies to detect latent pathogens should be conducted.

5.2 SAFEGUARDS IN PLACE THAT REDUCE PEST RISK ASSOCIATED WITH IMPORTED GERMPLASM

5.2.1 SAFEGUARDS: AT THE ORIGIN OF THE GERMPLASM.

Phytosanitary Certificate. The phytosanitary certificate is a safeguard established under the IPPC. The certificate is a statement by the exporting country that the U.S. quarantine requirements have been met and that the material was inspected within the 2-week period prior to export. PPQ keeps exporting countries posted as to regulations and phytosanitary requirements. The certificates issued by some countries have a higher credibility than those issued by other countries based on past records of violations. The United States does not accept the certificate as a sole safeguard upon which entry status is based. The certificate is useful in confirming the geographical origin of the import which is related to hazards and risks.

Inspection at Origin. Inspection at origin is an important safeguarding concept. Pests can be detected before the plant material is shipped rather than after the plant material is received. This reduces the chance of pest dissemination from a U.S. port of entry. While it is the responsibility of the importing country to protect itself from the threat of extra pests, there is now a trend by importing countries to place more of the burden of inspection on the exporting countries. The preclearance program is an application of the inspection at origin concept.

Most quarantine services, however, would not allow inspection at origin to replace inspection upon entry except under cooperative arrangements for commercial materials of low risk types. While inspection at origin might facilitate the entry of restricted germplasm, it would not help to lift constraints of postentry or prohibited entry (Departmental permits) because inspection is not an adequate safeguard for detecting obscure pests such as viruses, bacteria internally seed-borne pathogen, etc.

Treatment at Origin. In theory, treatments conducted at origin should have the same level of effectiveness as treatments conducted at entry. If the importing country could have confidence that the treatment was administered correctly, more weight could be given to such treatments. For commercial shipments, many importing countries send inspectors overseas to observe the treatment procedure. For germplasm in small quantities, on a case-by-case basis, treatments administered elsewhere have been accepted but this is not a frequent occurrence.

For restricted germplasm, there is not much to be gained by treatments administered elsewhere since the material must be inspected upon entry. For postentry and prohibited materials, there are usually no effective eradicant treatments except perhaps hot water, hot air, or formaldehyde in some pest/host interactions. While therapy of the mother plant may result in the production of progeny some of which are free of a virus, the progeny must be tested to locate the individuals which are free of virus. Contrary to popular beliefs, neither heat therapy nor tissue culture automatically free a plant of viruses. Both techniques usually increase the probability of obtaining clones free from viruses.

In essence, treatments at origin do not offer much promise in improving germplasm entry because conducting treatments, when effective, are not a limiting factor in germplasm processing.

Certification at Origin. Certification at origin is a useful safeguard for the importation of germplasm, but the procedure has had limited application. A clear understanding must be developed between the exporting and importing plant quarantine service because the certification discussed here extends beyond the routine phytosanitary certificate. Certification refers to a government sponsored or monitored program in place to detect and eliminate certain pests named in the program. To understand the meaning of "certified" one must know the range of pests covered by the certification. Certified material may be tested to be free of one or two pests or all important pests, or even "pest free" depending on the testing program. For postentry and prohibited germplasm, PPQ has accepted certification for commercial plants or germplasm as follows:

1. Prunus (cherry, plum, etc), Malus (apple), Pyrus (pear) Chaenomeles and Cydonia (quince) are prohibited from all countries except Canada. Consequently, germplasm must enter under a Departmental permit for passage through a quarantine station. An important exception is that germplasm from research stations in Germany (FRG), the Netherlands, Belgium, France, and the United Kingdom may be planted in the United States under postentry quarantine on the approved site of the importer. The plant protection service of the exporting country must attest on a phytosanitary certificate that the material meets U.S. requirements.

2. Syringa (lilac),which is prohibited from Europe may be imported into the United States from the Netherlands for direct planting if plants are certified to have been produced from virus-tested mother plants.

3. Dianthus (carnation) from any country, must be planted in postentry quarantine. However, Dianthus may enter as restricted, (e.g., requiring only inspection and treatment) if it has been propagated in the United Kingdom from mother plants indexed by U.S. standards and maintained in the greenhouse under a nuclear stock foundation program.

4. Manihot (cassava) is prohibited from all countries, but tissue cultures from CIAT (International Center for Tropical Agriculture, Cali, Colombia) may enter for direct planting if accompanied by a plant health statement as issued by CIAT and a phytosanitary certificate issued by the plant quarantine service of Colombia.

Pathogen Detection Tests at Origin. More high risk germplasm could enter the United States with less restriction if pathogen detection tests conducted at origin could be accepted as shown in the previous section. Since the germplasm is prohibited because it could carry pests of quarantine significance which cannot be inspected for at ports of entry, obscure pathogen detection tests at origin would facilitate entry of such germplasm.

There are advantages in conducting tests at origin. There would be less chance of pest escape in the United States if pests were detected at origin. Also, pathogen detection tests require "knowns" which are tests using a known isolate of the pest to be detected. If these tests were conducted in the United States, the known culture (i.e., exotic pests of quarantine significance to the United States) would have to be imported or could not be used if the known is particularly hazardous. The disadvantage is that while the mother plant tests are conducted at origin, some of which require 2 years, the progeny could become infected. The longer the time between the time the mother plant is tested and vegetative material or progeny is exported, the higher the chance is of the progeny becoming infected or reinfected. The disadvantages of detection at origin is that economic pressures from American nurserymen would be generated against certification at origin. Also, quarantine regulations would have to be changed by a revision of 7 CFR 319.37.

5.2.2 SAFEGUARDS UPON ENTRY OF GERMPLASM

Inspection. Inspection at the port of entry is an essential feature of the safeguards associated with the importation of germplasm--unless the burden has been shifted to the origin country by a preclearance or other special program. Inspection stations have been established at 14 ports of entry including Hoboken, Los Angeles, Miami, New Orleans, Seattle, and Honolulu. At these stations, the entire consignment or a sample is inspected depending on the plant genera and the country of origin. Inspection is limited to visual examinations for signs and symptoms with the aid of the light microscope. Seeds may be examined for insects by x-ray procedures. Inspectors are supported by identifiers and specialists in entomology, plant pathology, virology, and nematology.

The PGQC, an inspection station specifically for germplasm, has been established by PPQ in cooperation with the Plant Introduction Laboratory, ARS, USDA, at Beltsville, Maryland. PGQC handles only germplasm for export or import by the governmental agencies, universities, and research stations. The average turnaround time for imported germplasm is 1 day for perishable materials (plants, cuttings, tubers, budwood, etc.) and 3 days for seeds.

Treatments. Treatments are administered either because they are required as a condition of entry or because pests are found. Available treatments include methyl bromide fumigation, ethylene oxide fumigation (not at all stations), hot water treatments, and chemical treatments such as slurries, dusts, or dips. Usually, treatments administered at origin are not accepted, unless witnessed by PPQ officers because of problems associated with time/temperature/dose interactions. Treatment does not delay the entry of germplasm more than 1 day, but there may be phytotoxicity associated with the treatment. Unfortunately, there are not many eradicant treatments that kill all individuals of a pest species without harming the host. Usually, there is a zero tolerance, or near zero tolerance for the pest so that a treatment which may be effective at the farm level (e.g., 50 to 80 percent effective) would not be satisfactory as a safeguard against a pest of quarantine significance.

Permits. Permits are also an essential part of the safeguard system. The permit functions as a passport and facilitates entry through agricultural quarantine inspection. When the permit is issued, mailing or shipping labels are also issued. The label usually directs the consignment to an authorized port of entry of the permittee's choosing. A label on the outside of the mailed packages alerts quarantine inspectors at port offices.

The permit for most germplasm importations is referred to simply as "the permit." Postentry quarantine permits are issued for quarantine on the property of the importer while a Departmental permit is issued for prohibited plants whose entry is authorized under safeguard specifications. Permits for restricted material are usually written but may be oral for small quantities. However, Departmental and postentry permits are always written.

Pathogen detection test. Pathogen detection tests, other than light microscope or x-ray examinations, are not routinely conducted at inspection stations. Some locations such as the PGQC have authorization to rear out insects from larval or dormant stages to facilitate identification in another life stage. The PGQC has the capability of conducting seed health testing, the results of which may be used to determine entry status.

USDA Regional Plant Introduction Stations. Plant germplasm entering under the restricted category (i.e., subject to inspection and treatment upon entry) is frequently placed in greenhouse isolation by the ARS, USDA at one of the Regional Plant Introduction Stations although such isolation is not a quarantine requirement. These Plant Introduction Stations are located in Ames, Iowa; Geneva, New York; Experiment, Georgia; and Pullman, Washington. ARS plant pathologists conduct inspections and apply treatments as necessary to mother plants which will produce a second generation of seeds for distribution.

5.2.3 SAFEGUARDS: POSTENTRY QUARANTINE

Inspection, treatment, and permits. Plants entering under a postentry quarantine permit do so through a port of entry with a plant inspection station. Therefore, they are subject to the same inspection and treatments as restricted plants. However, in the case of imported fruit tree plants, a sample may be taken and sent to the Plant Quarantine Station in Glenn Dale, Maryland, for virus testing concurrently with postentry growing on the premises of the importor.

Isolation on the Property of the Permittee. After the consignment is inspected, it is forwarded to the permittee. The permittee has agreed prior to the receipt of plants that he will meet certain safeguard requirements such as site approval, distance from domestic plants of the same type, and labeling. The plants are inspected by Federal and/or State inspectors during the 2-year quarantine period. The constraints are that the imported plants must stay on the property of the importer unless written permission is received to move them. However, the permittee may propagate the accession and use the material for scientific purposes during the detention period.

5.2.4 SAFEGUARDS: PROHIBITED MATERIAL UNDER HIGH CONTAINMENT

Inspection, treatment, and permit. Plants which enter under Departmental permit are those prohibited to the general or commercial public. Departmental permits are issued for small quantities of plants imported for scientific purposes. Permits are issued on a case-by-case basis. Safeguard requirements are specified on the permit. The number of Departmental permits issued between May 1984 and April 1985 for five prohibited crops is shown in Table 9.

Certification. Certification at origin as to specific pathogen testing is occasionally accepted as one of many safeguards. For example, budwood from a greenhouse-grown grapevine plant, in Germany (from where grapevine plants are prohibited), was admitted on the basis of virustesting by a German grapevine virus specialist. Other certifications which have been accepted are discussed in Section 5.2.1.

Containment Safeguards. Specific safeguards are prescribed to meet the level of risk posed by hazardous plant material whose entry is prohibited except under Departmental permit. For example, corn from prohibited areas may be planted for greenhouse growing only between November and April when corn is not growing outside of the greenhouse. Imported rice may be passed through greenhouse containment at anytime of the year in Beltsville, Maryland, which is more than 1,000 miles from the nearest rice. The premises and plants are subject to inspection by a plant quarantine officer and/or a designated plant pathologist who is a specialist in the diseases of the imported species. Prohibited wheat seeds from countries where the flag smut fungus occurs may be imported for greenhouse growing or to two field locations (in Arizona and Oregon) under specified safeguards.

Pathogen detention. Plants under Departmental permit safeguard requirements may be inspected or tested by a plant quarantine officer or a designated plant pathologist. If diseased plants are encountered, the shipment may be destroyed and the containment area decontaminated. If clarification or confirmation of the identity of the causal agent is required, State, Federal, or University specialists may be asked to participate.

5.2.5 SAFEGUARDS: PROHIBITED MATERIAL IN QUARANTINE STATIONS

The high risk plant genera must be passed through a quarantine station where pathogen detection tests are conducted. The principal Federal stations are located at Glenn Dale, Maryland, and at Beltsville, Maryland, where a new station is under construction. Cooperative Federal/State quarantine facilities are located in Riverside, California, and Gainesville, Florida, for citrus; and in Davis, California, Geneva, New York, and Corvallis, Oregon, for grapevines. ARS, in cooperation with APHIS, maintains a quarantine facility for sugarcane at Beltsville, Maryland.

Inspection. Inspection takes place upon entry of the imported material, usually at the PGQC, and during the detention period in greenhouse quarantine.

Treatments. Treatments are administered as a condition of entry for sugarcane (fumigation plus hot water) or as necessary for other prohibited genera depending on pest finds. During the quarantine period, preventive pesticide applications are made to ensure that not only are any emerging foreign pests killed, but domestic pests that may serve as vectors are also killed.

Permits. A Departmental permit is required for all prohibited plants in quarantine.

Pathogen Testing. Pathogen testings are conducted for all genera prohibited because of submicroscopic pathogens in quarantine. These are described in the section which follows.

6.0 PATHOGEN DETECTION PROCEDURES IN CURRENT USE

6.1 INTRODUCTION

For the purpose of illustrating procedures now used in detecting pathogens in imported germplasm, the tests used for the following types of germplasm will be described: (1) Low risk seeds that enter as restricted (almost all crops), (2) seeds that enter under Departmental permit such as wheat or rice, because they are otherwise prohibited (crops or genera marked with an asterisk in Table 8), and (3) vegetable propagations of plants that enter under Departmental permit because they are otherwise prohibited (genera or crops shown in Table 8 with emphases on fruit trees, grapevine, potato, sweetpotato, ornamentals, and cassava. The same approach will be used to discuss new technological applications in Section 9.0. Most of the pathogen detection tests are for obscure viruses and virus-like agents.

Detection and identification of viruses and virus-like agents require a number of independent tests. Each test produces an indication that an agent is present (positive) or absent (negative). Variables are controlled better in the laboratory tests than in the greenhouse and better in the greenhouse than in the field. However, in any test, there may be operational errors or the failure to provide optional conditions which could give false positive or false negatives. More weight may be given to a result that shows the test plant is positive for the presence of a virus than one which is negative suggesting the absence of a virus. The confidence in negative results increases with replication and by using several tests with different methods of detection. If results are negative for several tests, a conclusion may be drawn that the agent is absent.

However, there is a line that must be drawn after negative results are obtained. Continued attempts to prove the negative became academic and even philosophical. For example, if the only test conducted is a serological one and the results are negative, one cannot be sure that the test plant is free of the virus under test. However, if serological tests, electron microscopy, and indicator tests are repeatedly negative; one can conclude with more confidence that the test plant is negative. Further testing to prove the negative are not biologically justified.

The tests employed to detect obscure pests are discussed in general terms in this section. Advantages and disadvantages of most tests are discussed as are the constraints. The techniques used to detect a particular pathogen that does not occur in the United States is often dictated by the detection method that is most reliable for scientists in foreign countries where those pathogens occur. If only symptoms and graft transmisibility have been described for a particular pathogen in foreign countries, then these are the only techniques available—until research is conducted to improved detection.

6.2 SIGNS AND SYMPTOMS

Symptoms are manifestations in the host of the presence of a disease agent and as such they reflect a host/agent interaction. The agent may be biotic such as a pathogen or abiotic such as air pollution. A given symptom may be incited by more than one type of agent. For example, yellowing may be caused by viruses, virus-like organisms, insect or pathogen toxins, nutritional imbalances, genetic factors, or unfavorable light conditions. In some host/pest interactions the symptom may be diagnostic but this is not usually the case. Often the host is symptomless or the biotic agent is latent. Symptoms are useful in suggesting the presence of a virus-like agent, but they can only be used as part of a package that leads to the characterization and identification of an agent (See section 6.7). Except in a few cases where diagnostic symptoms are present, symptoms are unsatisfactory for pathogen detection in seeds.

Signs are manifestations of the agent by which the agent may be seen in one or more of its various stages or life forms. Signs include fruiting bodies, cysts, eggs, spores, mycelium, larvae, pupae, adults, etc. Although signs show that an organism is present, the life stage present may not be useful taxonomically in an identification of the organism. Where identification is possible, signs are an important part of the identification process particularly during inspection upon entry, but also after entry during the growing season. Signs are found on or in plants and seeds. For example, signs in a consignmentof seeds may consist of weed seeds, fungous sclerotia or fruiting bodies, spores, mycelium.

The advantage of signs as a diagnostic test is that they show that an organism is present. The disadvantage is that they are not necessarily related to the incitant of any symptoms observed unless proven by experimental testing.

6.3 HOST RANGE

The total of all plant species and cultivars that support multiplication of a specific pests is known as the host range. When virus or virus-like diseases are described in the scientific literature, the host range of the agent may be compiled from both naturally and experimentally infected plants.

The advantages of host range as a diagnostic tool is that it is useful in characterizing a virus or other pest. The disadvantage is that due to variations below the species level in both the host and pest, host range cannot be used as a definitive detection procedure except for specially selected indicators. (See section 6.7.)

6.4 SEED HEALTH TESTING

Seed health testing (Neergaard, 1977) is a procedure used to detect surface or internally seed-borne pathogens. In one method, seeds, sometimes surface sterilized, are placed on agar media or on moist blotter paper in Petri dishes. The plates are held at cool temperatures and often under blacklight (UV) which stimulates fungus sporulation. Sometimes after germination starts, the dishes are placed in the freezer overnight to pill sprouts. At the end of 7 days, the sporulation on seeds is examined under low magnification for growth habit and morphological characteristics. In addition, spores are examined under high magnification.

The advantage of this method for seeds is that detections and identifications can be made for seed pathogens that are present in or on seeds without symptoms using a minimum of equipment and laboratory space. The test requires 7 to 10 days to complete. Greenhouse space is not required. The disadvantages are that viruses, viroids and many bacteria cannot be detected. Not all hazardous fungi will sporulate on seeds in moist chambers. Only a sample of a consignment can be tested so the test may not be practical for certain exotic pests occurring at a low incidence and for which there is a "zero tolerance" or "near zero tolerance."

Another method of testing seeds for pathogens is the centrifugation test. A sample of seeds is shaken in water and the filtrate is centrifuged to concentrate any spores in a pellet or residue which is examined under the light microscope. The advantage of this method is simplicity of operation and relatively short period of time to process a given sample. The disadvantages are: (1) That the spores detected may be field contaminants which may not have come from the crop being tested (2) that usually only samples can be tested.

Sampling error may overlook small concentrations of pests. The method has been used to detect spores of the Karnal bunt fungus as contaminants on wheat seeds.

The seedling symptom is still another test based on sowing seedlings in a sterile substrate such as a rolled blotter or sand, incubating the seedlings in an artifical environment for 2 to 4 weeks, and examining the seedlings for symptoms. The conditions more closely resemble natural conditions than do other seed tests but only pathogens that attack seedlings are detected, consequently, the method has limited application.

6.5 GROWING-OUT TESTING

Another method for testing and safeguarding germplasm imported as seed is the growing-out test useful for some host/pest interactions. Seeds are inspected, and blemished or broken seeds of any type are removed. The seeds may be treated and then planted in the greenhouse often during the winter months. Sometimes germinating seeds which pass a moist chamber incubation test (6.1.7) are transplanted to the greenhouse. In either case, the plants are inspected throughout the growing season. Plants with symptoms are rogued. A second generation of seed is produced. If the incidence of disease is significant, a third generation of seed is produced in the same manner until the improved health status of the seed warrants field planting and increase.

The advantages of the method are that laboratory or greenhouse indexing tests are not required and time is not a constraint since often only one growing season (3-5 months) is required. The disadvantages are: (1) Greenhouse space is still required for growing another generation of plants and may become a limiting factor, (2) often pest risk consideratons require that the greenhouse procedure may be used only during winter months, (3) the methods are mostly applicable to low risk germplasm, and (4) cross-fertilization may be a problem in some crops.

The growing-out method is used for some germplasm species imported into the United States. ARS uses the method of planting low risk (i.e., restricted) seed for a wide spectrum of crops at the U.S. Regional Introduction Stations. Under Departmental permits, high risk corn and sorghum importations may be planted in the greenhouse but only during winter months in locations where domestic plants of the same species are not outdoors. The method is used for high-risk wheat seed importations from Karnal bunt and flag smut (smut fungi) countries, but a number of other safeguards are required both at origin and after entry. Rice, which is prohibited from all countries, may be imported (subject to hot water treatment) under either of two sets of safeguards. If the rice is planted in a nonrice-growing area greenhouse, planting of seed throughout the year is permitted. If the seed is to be planted in a rice-growing area, seeds must receive a seed health test (6.1.4) and pathogen-free seedlings transplanted to the greenhouse and a second generation of seeds produced. The entire process must be under the direction of an approved plant pathologist who specializes in rice diseases. Since there are no known seed-borne viruses in rice, greenhouse, and laboratory testing for viruses is not required.

6.6 TRANSMISSION

Transmission refers to the experimental or natural means or methods by which viruses, virus-like agents, bacteria, fungi, and other pests are moved to healthy plants resulting in an infestation or infection. Viruses and other agents may be transmitted by one or more of the following methods:

(1) Mechanical or sap transmission in which the sap or purified virus from infected plants is rubbed on to healthy plants, (2) plant-to-plant contact which is natural form of sap transmission, (3) natural root grafting, (4) grafting as

a horticultural practice, (5) through seeds and sometimes through pollen, (6) by vectors such or parasitic plants (e.g., dodder), fungi, nematodes, mites, thrips, mealy bugs, aphids, beetles, bugs, leafhoppers, planthoppers, and other insects, and (7) through the soil or vectors in the soil.

Transmission is an essential part of the characterization of pests and serves as the basis for developing detection methods and identification. While transmission is not a detection test in the same sense as are the other procedures described in this section, transmission can exercise constraints on the phytosanitary aspects of plant introduction. When a plant species is known to be infected or infested with an agent of unknown etiology, constraints on the importation of that species develop. For example, coconuts, Cocos nucifera are damaged by diseases or disorders of unknown etiology. Some governments prohibit coconut plants even by government services for scientific purposes (USDA 1933-1985). The United States does not prohibit such plants if risk/benefits analysis is favorable and quarantine safeguards are adequate to prevent the escape of any pests while the causal agent is characterized. Fortunately, it is often possible to import the same variety from an area where the disease is not known to occur and then pass it through quarantine.

Another constraint imposed by transmission methodology is that not all agents are transmitted by all methods. If an agent is transmitted mechanically there are fewer constraints in testing methodology than when agents are transmitted only by insects or only by grafting as is discussed in section 6.7 which follows.

6.7 INDEXING

Indexing is the use of a transmission or inoculation technique (See section 6.6) to move an infectious agent from a plant which may not be showing signs or symptoms to a plant or agar medium which will show signs or symptoms.

The plants used for virus indexing are called "indicators" while the plants used for other pathogen indexing are called "differential host plants" or simply "differentials." In culture indexing, a piece of plant tissue is tranferred aseptically to an agar medium to detect the fungi and/or bacteria which grow out from the tissue into the medium.

An important feature of the indexing test is the length of the incubation period. The incubation period is the time between inoculation of the indicator and the appearance of symptoms. The incubation period must be known for each virus/indicator interaction. If the indicator has not shown symptoms after the completion of the incubation period plus an added time period as a safety factor, one may conclude that the plant under test did not have the virus for which the indicator was developed, assuming there were no extenuating circumstances. Tests conducted under the proper experimental procedures will include a "known virus" obtained from a host of the same genetic background and physiological history as the unknown viruses and transmitted to the indicator at the same time to serve as a check on the length of the incubation period and on the environmental conditions for testing. When symptoms show on the "known" control, the incubation period has been completed.

Indicator plants are those species, varieties, or cultivars in the host range of the virus or agent which have been shown by research, replicated in time and geographic location, to be so susceptible that they can be depended upon to show characteristic symptoms under a variety of conditions when an agent is transmitted by natural or artificial means to the indicator plant. Indicators have been developed for most important viruses and other pathogens. Sets of indicators designed to detect all or most of the viruses or virus-like agents of each of several crops have been developed by working groups of virologists or virus disease workers throughout the world. Consensus has been obtained for indicators to detect viruses of stone and pome fruits, some fruits, Citrus, grapevine, legumes, and many important ornamental genera.

Indicators are used in combination with symptoms to detect viruses and other agents. The advantages are: (1) The indicator plant is considered to be sensitive, in that a relatively low concentration of virus which might be missed in a less sensitive laboratory test has the opportunity to multiply in the indicator host until it reaches a high enough concentration to incite symptoms in the indicator, (2) indexing may be the only detection technique available, particularly for a recently described virus or unknown agent, (3) laboratory equipment and personnel trained in its use are not required, and (4) more weight can be given to a negative indexing test than can be given to a negative laboratory test to support the hypothesis that the plant under test is free of the virus under test, and (5) indexing with indicators may detect new unknown or undescribed pathogens better than more specific laboratory tests. The disadvantages are: (1) Greenhouse space, often with controlled growing conditions, may be required and may become a limiting factor, (2) healthy indicator plants must be grown or purchased from reliable sources, (3) "a known control" requires the inoculation of the indicator with a "known" virus. Thus requiring, in most cases, the propagation of the virus and taking a risk on its inadvertent escape, establishment, and spread, and (4) the incubation period; i.e., time required between inoculation and the appearance of symptoms, may be long, delaying the release of the germplasm.

The indicator plants must be held past the known or estimated incubation period before one can say that based on the absence of symptoms in the indicator the source plant does not contain the pathogen. With herbaceous indicators, the incubation is at least 4 weeks. For woody indicators, the incubation period may be more than 2 years. If the woody plant is a fruit tree and fruit symptoms are a requirement for detection, the incubation period can be 5 years. At onetime, the incubation period for citrus was 5 years of greenhouse incubation plus 5 years of field incubation. However, improvements and changes in indicator plant species and varieties reduced the incubation period to about 1 year. The incubation period and requirement for greenhouse or screenhouse space are constraints to the germplasm importation program. The dependence of detection tests on indicators with the resultant delays in releasing plants from quarantine have given the program a negative image among breeders or other importers who have liberal views about entry status and pest risk.

6.8 ELECTRON MICROSCOPY

Electron microscopy (EM) (Gibbs, 1976; Kurstak, 1981; Matthews, 1981; Milne, 1984) is an important aspect of virus characterization and is useful for detection. EM cannot be used to examine plant or plant parts directly. Instead either an epidermal strip, the exudate from leaves, thin sections, or extracts of plants are examined. Often partially purified virus is stained or shadow cast to improve images obtained on a fluoresecent screen, an electron sensor, or photographic emulsion. In addition, modifications such as scanning electron microsopy, freeze-dry etching, and ultra thin sectioning are available.

EM as a detection and characterization procedure may reveal the presence of virus in plants as well as particle size and shape which are characters used to place viruses in groups and to differentiate viruses from other parasites found in cells. Once EM has revealed particle size and shape and the virus has been assigned to a group, tests with indicators, methods of transmission, and choice of antisera can be narrowed down to those characteristic of the group. The leaf dip method can be used early in the detention period to determine whether virus is present (but is not accurate enough to determine that virus is absent).

The advantage of EM is that once the virus under consideration is placed in a group, it is possible to reduce the amount of testing since members of a group are often serologically related and may have common hosts or indicators as well as modes of transmission. If a determination can be made on an epidermal strip early in the detection procedure, it may be possible to focus in on a relatively small number of indicators and antisera for serological testing.

Once virus particles are detected in a foreign plant, even if the virus is not identified, testing could be terminated and therapy started or additional plant material requested from the foreign source. Early detection reduces the need for other tests. New plant material imported from another source would then be tested with a better chance of not being rejected because of exotic pests.

The disadvantages of EM are: (1) High cost of equipment, (2) requirement for trained personnel, (3) not as sensitive as the use of indicators for certain pathogens, (4) particles detected are not necessarily associated with the incitant of symptoms in a diseased plant, (5) a negative EM test does not necessarily mean that virus is not present, (6) viroids and viruses with small spherical particles in low concentration are difficult to detect with EM, and (7) very time-consuming, per sample examined. The advantages outweigh the disadvantages in so far as quarantine programs are concerned particularly when EM is combined with seriology. However, an EM is not part of the equipment in projects where germplasm in quarantine is tested. EM testing is usually done at a research location by nonquarantine personnel. Research laboratories usually limit the amount of "service" type of testing. In general, EM testing is not a routine part of the detection of pathogens in high risk germplasm importations.

6.9 INCLUSION BODIES

Inclusion bodies or cytopathological effects may be found in plant cells infected with viruses (Christie, 1977) (Gibbs, 1976; Hamilton, 1981; and Matthews, 1981). Inclusions can be observed by both light and electron microscopy. Various cytochemical and enzymatic tests can be conducted and observed by light microscopy. Viruses in nine virus groups produce inclusions which are diagnostic to the group level. In addition, some individual viruses produce other diagnostic structures. Sometimes, however, the inclusions are produced in very restricted tissue such as citrus tristeza virus inclusions in the leaf abscisson zones (Christie, 1977).

The advantage of inclusion body detection procedures are: (1) Early detection of some viruses may be obtained with light microscopy, (2) personnel can be trained easily using standardized procedures, (3) greenhouse space or complex laboratory equipment are not required, (4) some tests are diagnostic while others at least detect the presence of a virus, and (5) results can be obtained early in the quarantine period thus permitting reintroduction of the germplasm from another source.

The disadvantages of using light microscopy are: (1) Not all viruses or virus groups produce inclusions, (2) a negative result does not necessarily mean a virus is not present in the plant under test, and (3) processing a large number of samples requires personnel, time, and laboratory space.

Examination for inclusion bodies is not usually employed as a test procedure in locations where prohibited plants are in quarantine. There are not enough personnel or space available to utilize this procedure. When electron microscopy tests are conducted, observations for inclusions may be undertaken during routine examination for virus particle, but imported germplasm is not routinely examined by electron microscopy nor by light microscopy for inclusions.

6.10 SEROLOGICAL TESTING

Serological testing is one of the most important components of a pathogen detection program. It is most frequently used for viruses in plant introduction and quarantine programs, but not often for mycoplasma or bacteria. The basic tool is the antiserum used to detect the presence of a pathogen. Antisera are produced by injecting a partially purified virus or other pathogen (antigen) into an animal, usually a rabbit, which results in the production of antibodies by the animal. The antibodies are collected by precipitation from the globulin component of the animal blood serum. Tests are conducted in vitro when the antibody collected from the animal is mixed with extracted sap or purified preparations from a plant to be tested to determine by means of a characteristic reaction whether the plant is infected with the same agent as was injected into the rabbit. A complete test contains a number of controls including a "known" source of antigen to confirm that the test conditions are optimal (Clark, 1981, 1984; Gibbs, 1976; Hamilton, 1981; Torrance; 1981).

A positive reading for a serological test occurs when antigens (extracted from the plant under test) and antibodies react to form a precipitate. Several techniques have been developed to enhance the visualization of the positive reaction. As the enhancement of visualization increases, the sensitivity of the antiserum (ability to detect smaller amounts of virus) increases. In addition to sensitivity, specificity is an important part of antisera development. Antisera that are too specific may not detect all the strains of a pathogen. A highly sensitive antiserum may not detect all strains if the rabbit has not been injected with various strains of the virus. An antiserum which is not too sensitive may detect all strains but may fail to detect them at very low concentrations. An antiserum must be produced for each virus to be tested. Antisera have not been prepared for all viruses or virus-like agents, particularly those that have not been partially purified. Not all viruses are good antigens; consequently, effective antisera have not been developed for all viruses.

Among the serological tests which have been developed are the following: (1) Precipitin tests in liquid or agar, (2) agglutination tests, (3) microprecipitin or microagglutination tests, (4) agar double-diffusion precipitin or immunodiffusion tests with or without additives or sonification, (5) complement fixation, (6) bentonite or latex flocculation, (7) enzyme-linked immunosorbent assays (EIA or ELISA), (8) radio immunosorbent assays (RIA), (9) immunosorbent electron microscopy (IEM), and (10) immunofluorescent microscopy.

The advantages of serological tests are as follows: (1) Results may be obtained in a few minutes or hours or overnight, (2) the tests are specific, in that the agent may be detected amidst impurities, host material, or other unrelated agents, (3) greenhouse space is not required, (4) the tests may be conducted early in the quarantine detention period, (5) tests may be used for viruses or other pathogens which are not sap- or mechanically-transmitted, (6) some serological procedures are more sensitive than many other types of laboratory tests, (7) only a small amount of plant tissue is required, (8) antisera can be stored over a long period of time, (9) antisera can be exchanged between countries without moving the pathogens, (10) commercial diagnostic kits are available for some pathogen/host interactions, and (11) scientists are willing to exchange antisera.

Disadvantages of serological tests are as follows: (1) Antisera for many pathogens of quarantine significance have not been developed, (2) the research leading to the development of a useful antisera is costly and time-consuming, (3) known controls either require the importation of exotic pests which may not receive quarantine approval or else chemical inactivated pathogens, such as formaldehyde-treated virus, must be obtained, (4) some antisera may be too specific to meet regulatory needs, (5) the antisera developed to a specific virus occurring in one area may not be useful to detect other strains or concentrations of the same virus from another area, (6) false positives and negatives are possible, and (7) a serological laboratory and trained personnel must be available.

Serology is used in conjunction with indexing to detect pathogens of quarantine significance in vegetatively propagated prohibited genera at the PPQ facility. The tests in use now are microprecipitation, and double-diffusion in agar (occasionally), and ELISA using antisera developed by other quarantine services, donated by scientists, or purchased in commercial diagnostic kits. Plants that test positively by serological testing are not listed further. If the detection is early enough, plant materials may be reintroduced prior to the indexing tests or therapy begun. Plants that test negatively by serology are still tested by indexing. Serology can facilitate introduction of germplasm as a screening prior to indexing. Serology may be used to replace some indexing tests for some host/virus interactions where the tests are well developed, particularly for agents that are not of quarantine importance.

Serology is not used in conjunction with either low or high risk seed importations of germplasm, although techniques are available to test seeds. Most of the seeds on the prohibited list do not transmit seed-borne viruses of quarantine significance to the United States.

6.11 POLYACRYLIMIDE GEL ELECTROPHORESIS (PAGE)

The PAGE test is a procedure for detection of nucleic acids in small amounts of host tissue. The method consists of extracting various ribonucleic acids (RNAs) from plant tissue, partial purification of these RNAs, separation of RNAs by gel electrophoresis, and staining of nucleic acids which have migrated in the agar columns or slabs. Bands, developed from healthy and diseased extracts, are compared.

The presence of double-stranded RNA in plant extracts is considered to support the hypothesis that the plant is infected with a virus or virus-like organism. However, the significance of RNAs in such cases is controversial. Even so, some governments exclude plants if double-stranded RNA´s have been reported in certain plant species such as in the case of avocados.

The advantages of the technique are that it is specific provided developmental research has established the banding pattern for healthy tissue and known viruses. Thus, if an extract of a plant under test produces a column with bands matching a known virus, it may be concluded that the plant under test is infected with that same virus. However, the procedure is not routinely used since additional research is needed and negative results are not definitive.

6.12 NUCLEIC ACID HYBRIDIZATION

Viruses and virus-like organisms may be detected by nucleic acid hybridization procedures (Huang, 1984; Hamilton, 1981; Matthews, 1981; Owens, 1981). The principle involved is a comparison of the degree of base sequence homology for the constitutive purine and pyrimidine base of the nucleic acids between reference strains and test strains. The procedures, also referred to as molecular probing, include the blot testing, nucleic acid spot hybridization, and membrane filter hybridization. For example, potato importations are tested by the PPQ Plant Quarantine Station, in cooperation with the ARS Virology Laboratory, Beltsville, Maryland, for potato spindle tuber viroid by this method. The procedure consists of the hybridization of viroid RNA in the test sample with highly radioactive complementary DNA (cDNA) on a nitrocellulose membrane followed by radiographic detection of the cDNA-RNA hybrid. At the USDA Plant Quarantine Facility these procedures are not currently used with other pest/host interactions although the useful cDNA probes could be constructed in specialized laboratories.

The advantages of the technique are that it is highly sensitive and has the capability to detect strains. There may be more detectable nucleic acid in a plant then there is antigen needed for seriological tests. The disadvantages are that the preparation of diagnostic materials requires extensive research and development and complex laboratory equipment and procedures. Probes are available for ubiquitous agents that are easy to work with in plants. Probes are not available yet for many of the pathogens of quarantine interest. Many exotic pathogens occur in countries where sophisticated laboratory equipment is not available. Consequently, probes have not been developed. In other countries, only small amounts of probes are available for scientific use--and not available for quarantine use.

The procedure shows promise of being the most important tool for obscure pathogen detection. When more data and experience have been acquired, the procedure might replace indexing, at least for certain pathogens. A research program is underway by ARS at the U.S. Plant Introduction Station (where the PPQ Plant Quarantine Facility is located) to detect pathogens of quarantine significance in high risk germplasm such as Malus (apples), Manihot (cassava), and other genera by this method. This topic is discussed in more detail in section 9.15.

7.0 STATE QUARANTINES AND GERMPLASM

7.1 GERMPLASM IMPORTATION

Insofar as germplasm importations are concerned, Federal quarantines take precedence over State quarantines. States cannot restrict foreign germplasm to a greater extent than do Federal quarantines. Thus, a foreign plant that is enterable into the United States cannot be excluded by a State quarantine. However, Federal quarantines often take into account State positions. Some States have quarantines which prohibit or otherwise restrict the entry of pests or plants from other States.

States do have problems about the entry of plants in general and nursery stock in particular. Not all pests in the United States are distributed in all States. The States which do not have certain domestic pests may regulate against those pests. If a State has a regulatory program or quarantine in place, the Federal regulations are often geared to support those regulations even though the pest may not be of quarantine significance at a Federal level. Some States with high phytosanitary standards require treatments before certain materials enter these States.

7.2 STATE CERTIFICATION PROGRAMS

Many States have well developed certification programs which include obscure pathogen detection testing. The programs are voluntary, but nurserymen who wish to produce pathogen-tested stock may participate. Usually, the pathogen-tested mother plants are produced or held by agricultural experiment stations, various State agencies, or organizations that maintain nuclear stock or foundation stock. Propagative materials are sold to nurserymen. In various States, plants of potato, citrus, grapevine, stone fruits, strawberry, blueberry, raspberry, and ornamentals are certified to be free of one or more pathogens. Some programs cover certified seed such as beans and peas. These programs have been sucessful in eliminating the occurrence of certain pests in certain areas of the United States, thus creating pressure for more regulation of importations.

7.3 FEDERAL/STATE REGIONAL REPOSITORES

Federal/State cooperative programs financed in part by the CSRS, USDA, establish pathogen-free repositories. For example, the IR-2 Project for tree fruits has established a repository in the State of Washington, in a dry area under irrigation. The mother trees were pathogen-tested at Prosser, Washington, and an active stock serving as budwood sources is maintained in screenhouses at Prosser. The trees are retested each year. Tests include indexing, serology, and other laboratory tests. The IR-1 Project has established a similar program for Solanum (potato) species at Sturgeon Bay, Wisconsin.

7.4 USDA/STATE CLONAL REPOSITORIES

USDA and cooperating States have developed a clonal repository system for fruit and nut crops at various locations near agricultural experiment stations. Germplasm is being collected and conserved at these stations. These repositories are developing a pathogen detection program, but they may involve local specialists in some testing. The curators of some collections and the horticulturalists and breeders who they serve have problems about the length of time required to pass certain fruit genera through quarantine. They have proposed that repositories be allowed to do the foreign pathogen testing that is now done at quarantine stations.

The disadvantage of these proposed programs is that often the indexing would take place near commercial fruit-growing areas. Should a foreign pathogen escape from quarantine or a domestic vector acquire the foreign pathogen, the commercial production would be at risk. Clonal repositories would need greenhouse quarantine facilities, trained personnel, and laboratory equipment which should at least equal the safeguarding capacity of existing facilities. The question has been raised on cost/benefit consideration as to whether it is better to build new facilities and create new positions at three to six locations, or to expand present phytosanitary facilities which are not in fruit and nut crop-growing areas and where trained personnel are now located.

7.5 GERMPLASM EXPORTS

States played an active role with the Federal government in germplasm exports. Germplasm may originate in State certification programs, in foundation or nuclear stock programs, or in repositories where pathogen tests are conducted.

Many States are active in the inspection that leads to the issuing of phytosanitary certificates. PPQ, APHIS, has certified (based on education, experience, and training) certain State inspectors to make some of the inspections upon which Federal phytosanitary certificates are issued under the IPPC. Some countries even accept State certificates. Most countries require growing season inspection for germplasm imported into their country. Some accept the results of local certification programs for pathogen testing. Under the Federal/State cooperative program, regulatory officers can assist exporters in meeting the phytosanitary requirements of importing countries. If these requirements are not met, a phytosanitary certificate is not issued.

8.0 DATA BASE PROGRAMS

8.1 MANUAL DATA BASES

Over the years, plant quarantine services of many countries have accumulated data about domestic pests that require inspection for export certification and exotic pests that threaten their agriculture. The data in the past has been acquired by manual searches of the literature and is often maintained on cross-referenced index cards.

The plant quarantine services often publish circulars about these pests and all such services publish regulations in their own languages. The regulations of all countries translated into English are being assembled for publication by FAO. Digests of foreign regulations in English have been published by USDA (USDA, 1933-1985). The European and Mediterranean Plant Protection Organization (EPPO), has published circulars about pests (EPPO, 1981-1985) and regulations of member countries (EPPO, 1984).

In addition, much information has been assembled by individual scientists at universities, agricultural experiment stations, commercial organizations, and government agencies.

In spite of the fact that much of the collected information is available at many locations, it is not accessible to others except by a one-on-one contact or exchanging reference materials.

8.2 COMPUTERIZED DATA BASES

As far as the scientific literature is concerned, there are several computer readable databases available, particularly in developed countries (Kahn 1984b). In 1982, the American Society of Information Science (ASIS) listed 773 databases some of which cover agricultural and biological areas. Perhaps, the most widely used databases are AGRICOLA (based on the U.S. National Agricultural

Library holdings), BIOSIS (based on Biological Abstracts), and DIALOG (based on many databases). DIALOG is available to any country in the world that has telephone service and a suitable computer terminal. However, DIALOG is available to most countries only for access to bibliographic information.

The FAO is attempting to approach informational needs for agriculture including plant protection and quarantine. The FAO system, whose database started in 1975, is known as AGRIS (International Information System for Agricultural Sciences and Technology). AGRIS is available to about 100 countries who contribute information. AGRIS produces two products: (1) Agrindex, a monthly publication containing about 11,000 entries arranged by commodity, and (2) AGRIS magnetic tapes. All countries can use Agrindex and those with access to a computer can use AGRIS magnetic tapes. The U.S. National Agricultural Library, which is the North American representative to AGLINET (Worldwide Network of Agricultural Libraries), subscribes to Agrindex but not to the AGRIS magnetic tapes. Due to differences in time zones, the ability for the United States to participate is limited and costs are high. Consequently, the National Agricultural Library has discontinued its access to these tapes.

There is duplication in various databases, but since no producer or vendor has exactly the same set of sources, it may be necessary to accept duplication in order to broaden the coverage. Significantly, about 2 percent of AGRIS input comes from nonconventional sources (in-house unpublished information such as index card data furnished by some contributors). Consequently, information useful to plant protection and quarantine might be available in AGRIS and not in other databases. In addition to its offices in Rome and Vienna, AGRIS has regional offices in the Philippines, Luxembourg, Costa Rica, and Senegal for input and inquiry.

The problem associated with computerized databases is that they cover, at most, the past 15 years. However, the data needs for quarantine officers cover the past 50 years. Old reports about the occurrence of pests and their geographic distribution are essential. Consequently, both manual and computerized databases are required.

9.0 PROBLEMS, SOLUTIONS, AND RECOMMENDATIONS TO IMPROVE THE PROCESSING OF HIGH RISK GERMPLASM

In section 5.0, the regulatory aspects of U.S germplasm introduction were discussed for four categories of hazard or risk: (1) Preclearance, (2) restricted, (3) postentry quarantine, and (4) prohibited. It was emphasized that most germplasm falls into low risk categories. As long as it passes inspection upon entry at U.S. ports of entry, such germplasm enters the United States with little or no restriction or constraints. The category of postentry does pose a constraint in that the plant material must be detained on the property of the importer for 1 to 2 years. However, the importer may use the material for scientific or other purposes. Constraints and problems do exist for the genera that are classified as "prohibited" but which may be imported for scientific purposes under specified safeguards. Although these genera are relatively few in number, they are of significant economic importance to U.S. agriculture. Many of the constraints have already been addressed earlier in this report.

Most of the delays, constraints, and problems concerning the importation of plant germplasm are found in the group of prohibited deciduous tree fruit genera and a few other vegetatively propagated genera which must pass through quarantine to detect obscure plant pathogens. The group includes: (1) Prunus (plums, peaches, cherries, almonds, etc.), Pyrus (pears), Malus (apples), Cydonia (quince), Solanum (potato), and Ipomoea (sweetpotato), all of which are processed at the PPQ Plant Quarantine Facility, Glenn Dale and Beltsville, Maryland, (2) *Citrus* which is processed at the Glenn Dale facility and in Gainesville, Florida and Riverside, California, under a cooperative Federal/State program, (3) *Vitis* (grapevine) which is processed at Glenn Dale, Maryland; Davis, California; Geneva, New York; and Corvallis, Oregon, under cooperative Federal/State programs, and (4) *Saccharum* (sugarcane) which is processed in quarantine at Beltsville by ARS in cooperation with PPQ.

Since most of the problems and constraints fall into the fruit genera as well as a few other vegetatively propagated genera, this group will be used as a basis for discussing problems and then making recommendations. Some of the problem areas, as noted below, have constrained the movement of germplasm in the past. However, recent improvements made by ARS or PPQ, APHIS, or both agencies, have eliminated constraining problems or at least led to improvement. These are included in this section since they have been constraints in the past.

9.1 PROBLEM AREA 1

U.S. quarantine regulations impose delays and constraints on the importation of certain high risk plant genera. These genera, which are enterable only for scientific purposes, must be subjected to obscure pathogen detection tests under quarantine safeguards. The tests, particularly for woody plants, may require 2 to 4 years.

9.1.1 Discussion and Solutions

One solution is to reevaluate the pest risk status of certain pest/host interactions to determine whether recent developments in research or new information about pest distribution and biology warrant any changes in entry status. Can certain genera be moved from "prohibited" to "postentry" or from "postentry" to "restricted?"

An evaluation of regulations is underway, but the evaluation falls into a 5-year cyclic review. However, the evaluation competes with a wide spectrum of other PPQ regulations also under review. A review of all fruit and nut crop genera is underway, but present workload restrictions limit the review to three genera per year. However, it is projected that at least some fruit and nut crop genera can be taken out of postentry quarantine.

More emphasis might be placed on certification at origin after an evaluation of facilities and procedures conducted in locations where advanced detection methodology has been developed and applied elsewhere. Perhaps, approved foreign plant pathologists or virologists could certify as to the health status of their own lines or varieties. These evaluations require onsite visits and the time of a PPQ virologist but are presently not part of the routine assignment of PPQ personnel. Some evaluations have been made in the past on a case-by-case basis, but there is no organized effort to seek such sources.

In order to consider approving a certification scheme at origin, PPQ would request detailed information on the selection, maintenance, pathogen indexing, and certification of the parent plants of the scion wood and the rootstock. This information must include the frequency and method of testing of the parent stock, names of indicator plants and the agents they detect, the number of generations between parent stock and the propagating material to be exported to the United States, and the methods used to prevent the spread of pollen-borne, soil-borne, and nematode-borne pathogens. For example, for fruit trees, PPQ would require that these genera be tested by procedures recommended by the International Committee for Cooperation in Fruit Tree Virus Research. However, there are technical and regulatory problems associated with certification at origin which are beyond the scope of this report.

9.1.2 Recommendations

(1) PPQ, APHIS, should reevaluate the entry status of all prohibited and postentry genera. If workload problems are limiting factors, the analysis should be contracted out or the staff expanded.

(2) PPQ, APHIS, should consider certification at origin provided standards can be met.

9.2 PROBLEM AREA 2

Plant importations may not be established or their release delayed because of horticultural problems.

9.2.1 Discussion and Solutions

Some of the delays in the release of germplasm are due to the problems related directly to plant propagation and other horticultural matters. Vegetative materials often arrive in poor condition after delays in transit or the material arrives out of season and must be placed in cold storage. Sometimes material arrives which, although unsolicited, is nevertheless important. Rootstocks for propagation may not be available. There should be more advanced planning and coordination by all groups that participate in plant introduction activities.

Plant germplasm in a wide spectrum of plant families is imported from temperate, tropical, and subtropical regions. Plant propagators must be familiar with modern techniques of plant propagation for the wide spectrum of plant importation. These techniques should include tissue culture in order to ensure the survival of imported perishable material, particularly when it arrives in poor condition. Since 1957 when the pathogen detection program was initiated, plant propagation success was correlated with the presence of a professional plant propagator or horticulturalist. During periods when the position was vacant, there was an increase in loses due to propagation failures. Such losses resulted in delays for the importation of germplasm.

9.2.2 Recommendation

(1) Brochures should be prepared jointly by ARS and APHIS to inform scientists about how to collect, package, and ship perishable germplasm so as to minimize horticultural loses. A new brochure describing the quarantine aspects of plant germplasm importation should also be prepared. Brochures should be included with each permit as well as sent to members of relevant professional scientific societies.

(2) USDA and ARS committees on plant germplasm and genetic resources such as the National Plant Genetic Resources Board should have an APHIS representative as an observer at all open meetings. The name of the representative should be placed on the mailing list for documents generated by the Board (e.g., minutes of meetings).

(3) APHIS should be advised as far in advance as necessary to enable the Plant Quarantine Facility to gear up to planned explorations, collections, or transfer of germplasm collections.

(4) Departmental permit holders should have a requirement on their permits to notify PPQ, APHIS, in advance when they expect to import "prohibited" or high risk germplasm as listed in CFR 319.37(2) (USDA, 1984).

(5) The staffing for introduction/quarantine activities should include a well trained plant propagator at the sub-professional level. (Note: A recent reorganization at the Plant Introduction/Quarantine Station has given a high priority to staffing for horticultural and plant propagation. The subject has been brought up in this review because of past problems).

(6) Encourage the use of tissue culture for the importation of germplasm for those prohibited genera where shipping tissue cultures is more practical than shipping perishable plant material.

9.3 PROBLEM AREA 3

Lack of greenhouse and screenhouse space is often a limiting factor

9.3.1 Discussion and Solutions

Since 1957 when the pathogen detection program was initiated, there has usually been a shortage of quarantine greenhouse and screenhouse space. In some years, all plant introduction could not be accommodated and processed in a timely fashion. Greenhouses at the Glenn Dale location deteriorated over time inspite of some reconditioning. Available screenhouse space was reduced by deterioration at a time when maintenance funds were not available. The deterioration has resulted in a reduction in safeguarding effectiveness. New and modern greenhouse space is required.

9.3.2 Recommendations

Build new greenhouse and screenhouse facilities with appropriate safeguard features. PPQ has already constructed four new screenhouses required for the quarantine of deciduous fruit trees and seven new greenhouses at the Beltsville location. A total of nine greenhouses will be operational by mid-1986. These greenhouses and screenhouses are only sufficient to accommodate the USDA germplasm program for "prohibited" genera at its current rate of introduction. Consequently, additional greenhouse space will be required to accommodate any significant increase in the volume of introductions.

9.4 PROBLEM AREA 4

Lack of greenhouse environmental controls may reduce space available for processing certain prohibited plants.

9.4.1 Problems and Solutions

Plant material is imported from tropical, subtropical, and temperate regions. In order to maintain these importations, the quarantine station must not only have adequate greenhouse space, but it must have the flexibility to control the climate in each greenhouse section.

At the Glenn Dale Plant Introduction Station where many of the greenhouses are more than 40 to 50 years old, the temperature in the greenhouses could not be controlled in each section. With circulated hot water heat, there was a gradient in greenhouse temperatures starting with adequate temperature in the section where the thermostat was located but dropping off in the distant sections so that the temperatures were too cold in the winter months.

9.4.2 Recommendations

Build new greenhouses with climate control. (Note: At the new Beltsville location, seven greenhouses have been constructed—each section with individual temperate controls. However, the capacity is not sufficient to handle future needs (See next section).

9.5 PROBLEM AREA 5

Pathogen detection tests cannot be conducted throughout the entire year at Glenn Dale and Beltsville, Maryland

9.5.1 Discussion and Solutions

Pathogen detection tests using indicator plants must be conducted under relatively cool greenhouse conditions (i.e., 60° to 78° F). If the temperature is too high (i.e., above 90° F for prolonged periods), symptoms on indicators may be masked. As a result, most indexing tests must be conducted between October and April because the greenhouses are not air-conditioned during the summer. Either more greenhouses must be built or air conditioning added to existing houses.

A comparison of the use of air-conditioned vs. nonair-conditioned greenhouses follows. If five greenhouses without air-conditioning were used 7 months per year (5 x 7 = "35 greenhouse months") the same greenhouses air-conditioned could be used 12 months per year (5 x 12 = 60 months). Air-conditioning would increase the capacity of greenhouse about 40 percent. If it is not cost effective to air-condition existing greenhouses, new greenhouses should be air-conditioned. Unfortunately, the costs are high. As a rule of thumb, if the cost of commercial greenhouses without greenhouse sections is used as a standard, the cost may be doubled to build a quarantine greenhouse with sections and without air-conditioning and tripled to build a quarantine greenhouse with sections and air-conditioning.

9.5.2 Recommendation

Construct an air-conditioned quarantine greenhouse as the tenth greenhouse in the series. During the 2 years following construction, conduct a cost/benefit analysis and compare the usefulness of a nonair-conditioned house (used 8 months per year) with an air-conditioned house (used 12 months per year). Use the data to determine whether future new greenhouses should be air-conditioned.

9.6 PROBLEM AREA 6

The Plant Quarantine Facility does not contain any high-containment (i.e., sealed) greenhouses.

9.6.1 Discussion and Solution

The seven greenhouses at the new Beltsville location (and the four greenhouses at the Glenn Dale location) were built as insect-proof greenhouses. As such, the Beltsville greenhouses offer the safeguard protection for which they were designed--for genera considered to be of high risk because of exotic viruses.

However, some genera listed as "prohibited" in CFR 319.37, such as genera in the conifers, are so listed because of the threat of exotic fungi. There is no adequate space available to quarantine imported plants where the risk is air-borne fungi such as rusts. Such space requires sealed and air-filtered greenhouses under negative pressure.

9.6.2 Recommendation

Build one greenhouse which is sealed (and necessarily air-conditioned) with negative pressure and other features which prevent the escape of air-borne fungi should they sporulate while in quarantine.

9.7 PROBLEM AREA 7

Delays in Indexing Due to Pathogen Detection Testing Requirements

9.7.1 Discussion and Solution

Germplasm processing is necessarily delayed while pathogen detection tests are conducted at the Plant Quarantine Facility. The release of plants in quarantine may be delayed by (1) tests with long incubation periods and (2) the necessity to observe the foreign plants for symptoms. A solution would be to determine whether some alternatives to indexing upon entry (which are now in use on a limited scale) could be expanded to facilitate the entry of germplasm.

9.7.2 Recommendation

Determine whether some of the following alternatives now in use on a limited scale could be expanded:

(1) Accept certification at origin for some or all of the testing that would have been done after entry.

(2) Allow the scientist to have part of the shipment under safeguard and isolation on his premises but still available for experimental use while the remainder is tested concurrently at the Plant Quarantine Station.

(3) Allow specialists in the United States to do virus detection tests under approved safeguards such as is now done for Citrus (California), Vitis (Oregon, New York, California), sweetpotato (North Carolina).

(4) For some genera, it may be feasible to employ heat therapy as soon as introductions arrive before pathogen detection tests are conducted. This procedure is now used for grapevine introduction. By doing so, only one testing is required. At the present time, an introduction is indexed and if a pathogen is found the introduction is given heat therapy, and then it must be tested again to see if any propagations are successfully freed of the agent. This requires two indexing tests and a doubling of the length of time the plant is in quarantine. This approach is useful when the virus test incubation is long and virus incidence is high. This approach is used for grapevine indexing at the University of California, Davis.

(5) Accept certification for germplasm from International Agricultural Research Centers that are a part of the CGIAR network (Kahn, 1982b; 1983b; 1984a). Under this proposal, consignments would arrive with a phytosanitary certificate from the plant quarantine service and a plant health statement issued by the International Agricultural Research Centers listing the tests that were conducted.

(6) Encourage the importation of tissue cultures as a safeguard against the entry of certain exotic pathogens. ("Tissue culture" is used in its broad sense to cover aseptic plant culture derived from such sources as birds, meristem tips, meristems, or other explants). However, indexing may still be required for obligate parasites such as viruses.

(7) Determine whether the "new detection methodology" (i.e., molecular probes) can substitute for indexing and, therefore, reduce delays and reduce the requirement for greenhouse space.

9.8 PROBLEM AREA 8

Negative results, as a safeguard, present regulatory problems.

9.8.1 Discussion and Solution

Negative results for a given detection test do not necessarily mean that the plant under test is free of the agent under test. There may be procedural or reagent errors that lead to false negatives. More confidence can be given to negative results if a series of different tests can be conducted. If the plant tests negative by indexing, staining, electron microscopy, and serology more confidence could be given to negative results. If molecular probes were developed for exotic pest detection, the problem of negative tests would be significantly reduced.

9.8.2 Recommendation

(1) Conduct a wider range of tests that is now possible with existing staff, equipment, and facilities.

(2) To do so would require an increase in budget to support new detection methodology.

(3) Conduct research (See 9.15) to support regulatory diagnosis activities or contract for the research.

9.9 PROBLEM AREA 9

For many genera, there is no rapid screening program to detect pathogens in introductions soon after their arrival.

9.9.1 Discussion and Solution

Current detection programs rely on indexing which is a time consuming process requiring greenhouse space. The amount of indexing could be reduced if introductions could be rapidly tested in a screening program designed to locate infected plants early in the detection period. If such plants were identified early, additional material could be introduced again from another source or infected material could be subjected to heat therapy. If "positives" were detected early, the introduction would not be placed in the indexing program.

The methods described earlier in this report could be utilized for primary screening. These methods include molecular probing, advanced serology, electron microscopy, and staining for inclusion bodies. However, these methods are not routinely employed in indexing programs at Glenn Dale or Beltsville.

ARS has started research at Glenn Dale on the application of new technology to pathogen detection testing with apples, cassava, and other crops. It is conceivable that because of increased sensitivity the new technology might replace indexing. However, before this could happen much more research would be required to develop probes that cannot be purchased or exchanged with other scientists.

Recent developments in pathogen detection using the "new technology" such as molecular probing or advanced serological procedures have demonstrated an increase in sensitivity and specificity. The author used the subject matter and abstracts of a recent symposium on new techniques for detecting viruses (Association for Applied Biology, 1985) to sample the potential application to detecting obscure pathogens in germplasm imported into the United States. The symposium consisted of 29 formal presentations and 40 poster presentations related to plant pathology or virology.

The 29 formal presentations, including surveys of concepts and procedures for various forms of molecular probing (spot hydridization, Western blotting, dot blot hydridization, sap spot hydridization, complementary DNA, several modifications of the ELISA technique, immunogold probes, protein A probes, monoclonal antibodies, polyacrylimide gel electrophoresis, and fluorimmunoassay. In the course of these presentations, 23 agents were mentioned of which 10 were of quarantine interest to the United States. In the 40 poster presentations at least 50 agents were mentioned, of which 28 were of quarantine interest to the United States.

Assuming this international symposium reflects the type of research being conducted in the use of new technology for pathogen detection, it is evident that research is underway in areas of interest to quarantine programs. Unfortunately, the research is in initial stages of development. Before these procedures are available on a routine basis, the work must not only be confirmed, but expanded to a wider range of agents and their strains than used in the current experiments. Some agents are undescribed and some exotic diseases or syndromes are incited by unknown agents.

9.10 PROBLEM AREA 10

Biological data and information storage and retrieval restraints.

9.10.1 Discussion and Solutions

Pest risk analysis is the foundation of regulatory decisions, positions, and policies, as well as regulations. Such analysis is based on the best available information. In either case, data must be acquired and stored for easy access in order for specialists to respond in a timely fashion to management needs in particular, and the protection of U.S. agriculture, in general.

PPQ, APHIS specialists on the Biological Assessment Support Staff (BASS) have requested the necessary computer hardware for dedication to biological data storage. Although management early on approved the concept, the acquisition had been delayed either by budget constraints, low priority, or the policy to channel all requests through nonbiologically based administrative units responsible for selecting hardware and vendors. Whatever the cause, at any point in time in the past, the hardware has not been available--until recently.

During 1985, PPQ purchased two IBM personal computers for use by BASS for its wide spectrum of data processing requirements. However, it has not been possible to staff for operators to process the data once it has been acquired from the scientific literature and other sources.

In addition, BASS has only one information specialist to acquire literature, to select the data that is useful for pest risk analysis, and assemble it in a package that may be used by a specialist in pest risk assessment. As a result, specialists must spend some of their time acquiring information.

The PPQ virologist at the Plant Quarantine Facility searches the literature and stores his data by manual methods. This requires the dedication of some of his time that might be better utilized in processing germplasm.

9.10.2 Recommendation

Give biological data acquisition and storage a higher priority within BASS by staffing for at least one computer operator and two additional information specialists. BASS currently has 11 specialists in entomology, plant pathology, mycology, botany, and virology, but only one information specialist.

9.11 PROBLEM AREA 11

ARS as APHIS responsibility for processing plant germplasm at the Plant Quarantine Facility

During the period between 1957 and 1972, the Plant Quarantine Facility at the U.S. Plant Introduction Station, Glenn Dale, Maryland, was managed by ARS with personnel from the Plant Quarantine Division and Crops Research Division. In 1957, the program was initiated and in 1971-1972, the USDA regulatory divisions were split out of ARS to form APHIS. Since 1972, the two agencies have both dealt with germplasm in separate roles. The facilities were retained by ARS, but space was assigned to APHIS. APHIS furnished four to five employees, purchased equipment, and allocated about $12,000 to $18,000 per year to the Glenn Dale station budget in exchange for the services of ARS personnel to conduct virus indexing of apples and pears. The budgets of the two agencies were separate. In some years, ARS was unable to provide funds for greenhouse maintenance. As a result, APHIS performed "patchwork maintenance," but in the end the greenhouses deteriorated.

One of the anonymous reviewers who was familiar with the ARS, APHIS program made the following comments, quoted in part as follows.

"The present system for prohibited plants has done a good job of excluding exotic pathogens but a poor job serving plant breeders because the system is very slow and inefficient. Many of the changes that are suggested in 9.2 are symptomatic of a neglected program. This neglect has resulted from the fact that two agencies, APHIS and ARS, have shared this program with no clear understanding of who in which agency is responsible for making the program work well enough to serve the public. Those involved from ARS have concentrated on research, the main activity of an ARS scientist, and those involved from APHIS have been involved with other regulatory problems. Consequently, no one manager has the activities analyzed in part 9 as his or her major responsibility. Instead, the plant material is passed from one group to another, from one agency to another and back, in a maize of steps between arranging importation and release from quarantine. This maize is created because each agency has its own interests in different aspects of the program but does not want to be responsible for the whole program. Since no one manager or agency is responsible for making the program work, either or both agencies can ignore the overall needs of the program and still avoid responsibility for the resulting deterioration."

9.11.1 Discussions and Solutions

In the author's judgment, the anonymous reviewer presented a criticism that accurately reflects the relative roles and the interaction between ARS and APHIS in the cooperative program to process germplasm through quarantine. Employees in each program were dedicated to their respective tasks and objectives in improving or protecting U.S. agriculture. The activities were cooperative but independent. The dichotomy adversely effected funding and other types of support.

APHIS and ARS recognized that there were problems at the working and support levels. In 1984, the ARS/APHIS Plant Germplasm Committee was formed. The ARS members of the Committee are G. Vandenberg, A. Stoner, and Q. Jones (recently retired), and the APHIS members are W. Helms, R. Williamson, and R. P. Kahn. The Committee met to set objectives and guidelines and charged a working group to develop a plant that could be sent forward concurrently by both Agencies for approval at higher levels within the USDA. The membership of the working group are as follows: From ARS; A. Stoner, B. Parliman, and C. Gabriele and from APHIS; R. P. Kahn and J. Foster.

The Committee and its working group developed a proposal which integrates ARS and APHIS personnel into a team. The team would be supported by funds from both Agencies. All existing germplasm introduction and quarantine activities would be carried out by the team under single leadership which would be identified as neither ARS nor APHIS, but as a national program. The author of this report who is also a member of the working group and the Committee is convinced that the proposed plan, if approved, would eliminate many of the constraints and delays that now affect the timely introduction of germplasm. The proposed plan is in the final stages of preparation but has not yet received administrative approval at higher levels in the Department.

9.11.2 Recommendation

1. The ARS and APHIS positions should be integrated into a single administrative unit that might be called the U.S. National Plant Germplasm Quarantine Center which would become a germplasm "campus."

2. ARS' research activities concerning the application of modern technology (molecular probes) to the detection of pathogens, including exotic pests of quarantine significance should be moved from Glenn Dale to property adjacent to the new Plant Quarantine Facility under construction by APHIS.

3. ARS activities concerned with plant introduction should be moved to the property adjacent to the new Plant Quarantine Facility.

4. The APHIS Plant Germplasm Quarter Center (PGQC) which is an inspection station for germplasm (Bldg. 320-E) should be moved to the new location. Such a move has been planned for more than 5 years, but funds ($1,400,000) have not yet been made available. The budgeting for construction of this building or some of its components should be accelerated. Although a new virology laboratory is in the FY 1986 construction budget, the PGQC has not yet been placed in any fiscal year budget.

5. Funding for ARS construction should be made available by FY 1987. The implementation of the germplasm team project depends on the ability of ARS to move its Glenn Dale staff to Beltsville to conduct both research and germplasm management activities proposed in the plan developed by ARS and APHIS.

9.12 PROBLEM AREA 12

Research on rapid diagnosis of exotic pests has not received high enough priority.

9.12.1 Discussion and Solutions

Within USDA, responsibility for agricultural research lies with ARS, although APHIS may support some research and participate in methods development activities. Each year APHIS presents to ARS a list of research projects needed to support regulatory activities. Many of these are undertaken and completed. APHIS conducts methods development activities in regulatory treatment and some practical applications of biological control, pest suppression, eradication, and containment.

ARS has established a position at Glenn Dale--which is part of the proposed new team to be moved to Beltsville in which a plant pathologist and technician are looking into molecular biology techniques for the detection of exotic pests of quarantine importance.

A reviewer of the draft of this report supported the concept that more use should be made of the probing techniques.

9.12.2 Recommendation

1. ARS should be given additional funds to expand its project to develop probes for the detection of exotic pathogens of quarantine importance. These funds should allow for an additional scientist or one or more post-doctoral fellows. Funds for new equipment needed to keep pace with this rapidly developing field should be provided.

2. The ARS construction budget as mentioned in section 9.11 should be funded is soon as possible. A molecular biology laboratory and supporting facility is needed on property adjacent to the new plant quarantine facility.

9.13 PROBLEM AREA 13

Changing U.S. quarantine regulations is a complex and time-consuming process which impacts on germplasm introduction

9.13.1 Discussion and Solutions

Changes in regulations, many of which were promulgated more than 30 years, are justified based on new biological data, changes in methodology, and updated pest risk analysis. Some of the changes might be more restrictive and some might be less restrictive as a result of biological updating.

Changes that are more restrictive are relatively easy to make as far as government procedures are concerned, particularly, when there is some urgency or emergency related to protecting U.S. agriculture. The more restrictive regulations may prohibit certain genera of plants. If so, commercial importations would be stopped and germplasm could enter under Departmental permit with appropriate safeguards. Although safeguards make it possible for the plants to enter, they necessarily also delay entry particularly if passage through quarantine is required.

Changes that are less restrictive are more cumbersome to make because usually a notice in the Federal Register followed by public comments or even public hearings are required. Changes that are less restrictive make it easier to bring in both commercial plants and germplasm. The increase in importation of commercial plants is of concern to interests outside of the scientific community. The interests of those outside of the scientific community interact with the regulatory process, and if delays result, the delay also affects the entry of germplasm by delaying the implementation of the less restrictive entry status regulation.

9.13.2 Recommendations

1. Develop, for the short term, an approach to administrative procedures in quarantine areas that allow APHIS to implement at least those less restrictive changes in genera of interest to scientists but not of concern to other interests.

2. Develop, in the long term, an administrative procedure that requires the government to go through the administrative procedures of the Federal Register, public comments, or public hearing for all prepared changes, either less or more restrictive, within 6 months of the date that the change has been proposed to the Administrator of APHIS.

9.14 PROBLEM AREA 14

"Cleaning up" Infected Plant Germplasm

At the present time, there is not enough technical support to "clean-up;" i.e., free plant importations, when possible, from the foreign agent that U.S. regulations are promulgated to exclude.

9.14.1 Discussion and Solutions

Pests and pathogens of quarantine importance have been detected in plant importations in quarantine (see section 3.1). Sometimes, it is relatively easy to import the same germplasm from another source, but often this option is not available (e.g., plants collected in the wild or special breeding lines). Therefore, a limited "clean-up" program has been instituted at the Glenn Dale and Beltsville sites. The "clean-up" procedure consists of heat therapy followed by meristem or meristem tip culture and then by virus indexing. The program has been used for virus infected potato and sweetpotato. However, the "clean-up" procedure has not been used with woody plant introductions, particularly, fruit trees. Before 1975, the clean-up of some fruit tree introductions infected with common viruses was conducted by cooperating scientists in Universities and Experiment Stations (e.g., Dr. George Nyland, University of California, Davis).

However, in the past 10 years, lack of technical support was a limiting factor. To adopt the technology for fruit tree tissue culture, some trials were conducted by a quarantine technician in cooperation with ARS and Beltsville, but the program failed to materialize when the technician was transferred to another project.

The proposed plant for integration of ARS and APHIS personnel should place the Plant Quarantine Facility in a position to use clean-up procedures for most of the genera in the introduction program.

9.14.2 Recommendation

1. Expand tissue culture and indexing to include woody plant species such as deciduous fruit trees. To do so would require additional technical support over that available at this writing.

9.15 PROBLEM AREA 15

Although recent advances in molecular biology such as probes, may solve future plant quarantine problems, their practical applications for the detection of exotic pests of quarantine significance is limited in the present and is likely to be so in the future unless the direction of research is changed.

9.15.1 Discussion and Solutions

Scientists immersed in molecular biology, including a reviewer of the draft of this paper, are quick to point out that molecular probes (section 6) can replace most, if not all of the diagnostic procedures discussed in section 6 of this report. The author concurred in this assessment for many host/pest interactions. The procedures can certainly reduce delays and constraints—but will they replace conventional tests altogether?

The following obstacles are in place to limit the application of biotechnology in the near future.

1. Some diseases, actually syndromes, are incited by agents of unknown etiology. The incitant must be proved to be infectious to be of quarantine interest which can only be done by indexing. Existing probes could determine whether the agent is one member of a group for which a probe has been developed. However, the probe alone will not establish the identity of a disease of unknown etiology. A negative test with an existing probe supports the hypothesis that the incitant is not the one for which the probe was developed. A positive test indicates that the plant contains an agent within the range of the probe, but

probes alone cannot establish that agent detected in the incitant of the syndrome.

2. Scientists develop probes to use as a tool in basic research or for use in diagnostic programs. In a given country, most probes are made for domestic pests and not for exotic pests.

3. Scientists in one country are not likely to develop probes for exotic pests in other countries unless quarantine restrictions for the importation of the pest are overcome by safeguards and the agent is of research interest to the importer.

4. The concept of "one-size-fits-all" (as developed in the hosiery industry) may be applied to probes but the concept may not necessarily meet quarantine needs. Under the one-size-fits-all concept, a probe can be developed to detect viroids in general. Such a probe would be useful since almost all viroids are of quarantine interest (because the agent does not occur in the importing country) or of economic importance (because a crop is undergoing a domestic "pathogen-free" certification program).

However, the concept is not meaningful for a group of "related" viruses which can be detected by a single probe or a group of related bacteria which might be detected by another probe--if the groups contain a mixture of exotic and domestic agents. It is not enough for the quarantine service to know that a plant is infected with a member of the group. United States quarantine officers take regulatory actions against pests which do not occur in the United States, or which are not widely distributed or under a Federal domestic quarantine, or in some cases, exotic strains of domestic pathogens.

Most countries do not require that imported plant material be pest free. To do so would stop the movement of plants.

5. The one-size-fits-all concept for probes--developed in one country

might not detect strains in another unless research has been conducted to determine whether those exotic strains have nucleic acid sequences in common.

6. An inquiry from another country asks for the U.S. position on the detection of double-stranded RNA in plants which is evidence that a plant virus is present. The nature of the question suggests that the entry status of a shipment of plants might be based on the presence of double-stranded RNA in a sample—even when the identify of the virus is not known.

7. Molecular biology at what ever level of development will not eliminate the need for quarantine greenhouses or some detention period in quarantine because (1) the plant must be grown and propagated in quarantine until the tests, whereever they are, are completed, (2) the plants must be grown to be observed for symptoms, particularly those incited by agents for which there are no probes, (3) infectivity tests must be conducted for those cases where probes have not been developed, (4) some exotic pests in other countries, particularly developing countries, will not have scientists available to develop probes.

In summary, the methodology is available to develop detection procedures that could significantly decrease quarantine restraints and reduce delays and even eliminate some virus indexing. However, the probes developed for the most part are not for those of quarantine interest to the United States.

9.15.2 Recommendations

1. The ARS program at Glenn Dale, Maryland, (to be moved to Beltsville) should be expanded to include additional personnel and funds to develop rapid diagnostic procedures using biotechnology and/or molecular biology.

2. ARS should consider allocating some of its biotechnology funds for practical diagnostic procedures and the production of probes at other locations, particularly for domestic pests with exotic strains of quarantine importance.

3. APHIS should be allowed to conduct applied research in diagnostic

areas or have funds available for research by contract.

4. Funds available in U.S.A.I.D., O.I.C.D., Public Law 480 and bilateral research programs should be channeled into molecular biology research leading to the development of probes for pathogens (including their strains) of quarantine significance which occurs in certain countries.

5. U.S. scientists should give grants such as sabaticals or temporary assignments to work in molecular biology and develop probes as suggested in 4.

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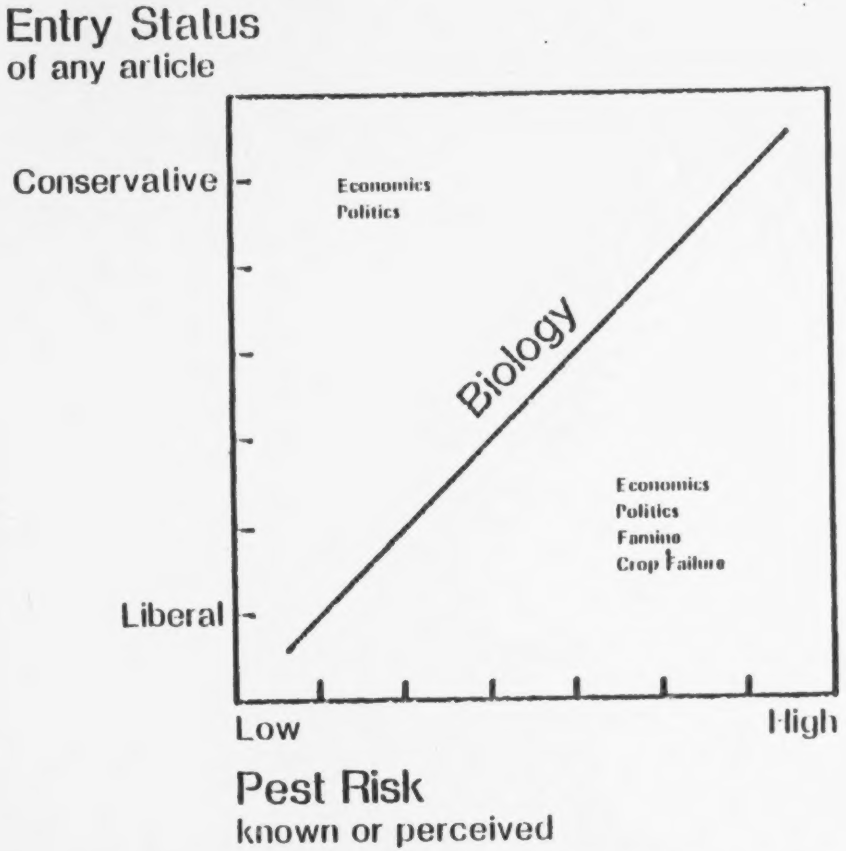

Entry Status
of any article
Conservative
Liberal
Economics
Politics
Biology
Economics
Politics
Famine
Crop Failure
Low
High
Pest Risk
known or perceived

Figure 1. A diagramatic representation of the relationship between pest risk (PR) and entry status (ES) of any article including germplasm. When PR and ES are matched; e.g., high pest risk and conservative entry status or low pest risk and liberal entry status, points developed by this matching falls on the biological curve. When PR and ES are mismatched; e.g., high pest risk and liberal entry status or low pest risk and conservative entry status, such points are not on the biological curve. The mismatching may be required in case of famine or crop failure but such positions are not biologically based with respect to the pest in question.

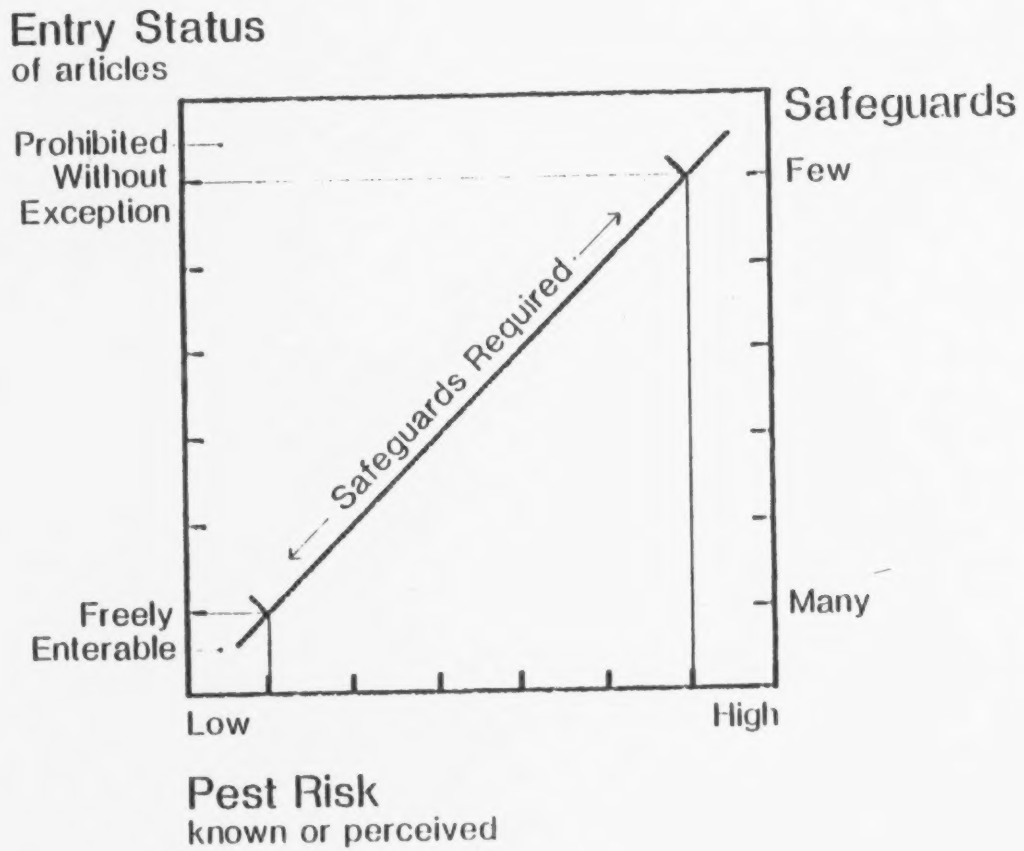
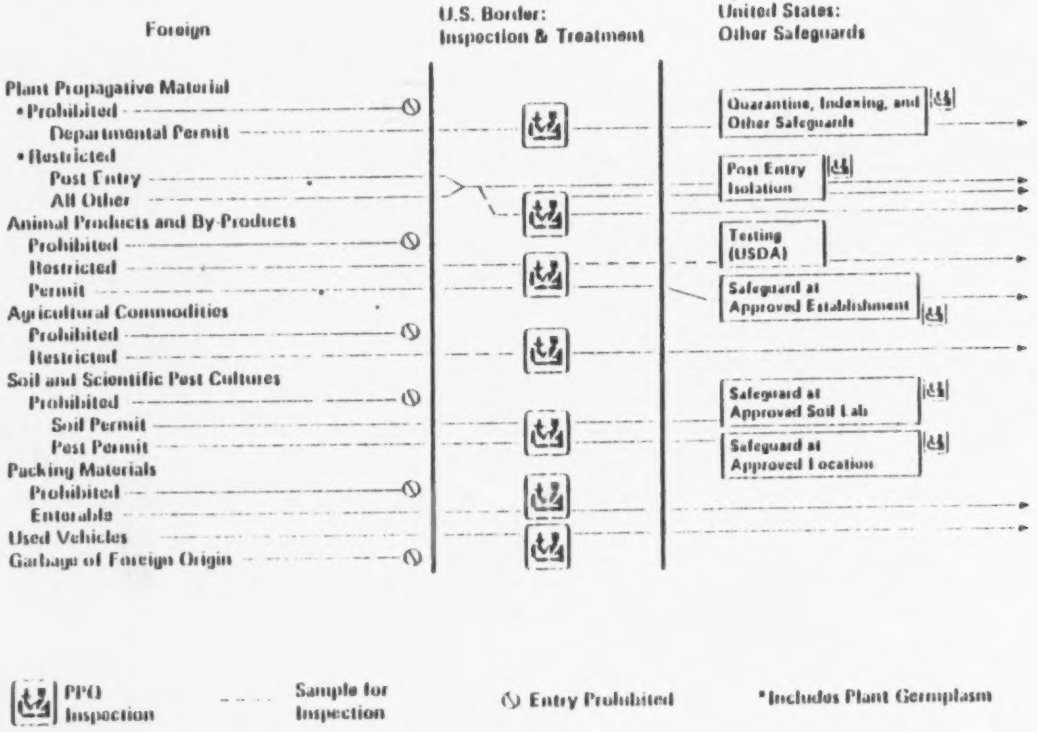

Entry Status
of articles
Prohibited
Without
Exception
Freely
Enterable
Safeguards
Few
Many
Safeguards Required
Low
High
Pest Risk
known or perceived

Figure 2. The use of the pest risk biological curve to illustrate the concept of safeguarding. As the number of safeguards is increased, entry status may become more liberal because pest risk has been lowered.

Figure 3. Regulatory Status of Foreign Articles Presented for Entry at U.S. Borders And Safeguards in Place After Entry


Foreign
U.S. Border: Inspection & Treatment
United States: Other Safeguards
Plant Propagative Material
•Prohibited
Departmental Permit
•Restricted
Post Entry
All Other
Animal Products and By-Products
Prohibited
Restricted
Permit
Agricultural Commodities
Prohibited
Restricted
Soil and Scientific Pest Cultures
Prohibited
Soil Permit
Pest Permit
Packing Materials
Prohibited
Enterable
Used Vehicles
Garbage of Foreign Origin
Quarantine, Indexing, and Other Safeguards
Post Entry Isolation
Testing (USDA)
Safeguard at Approved Establishment
Safeguard at Approved Soil Lab
Safeguard at Approved Location
PPQ Inspection
Sample for Inspection
Entry Prohibited
*Includes Plant Germplasm

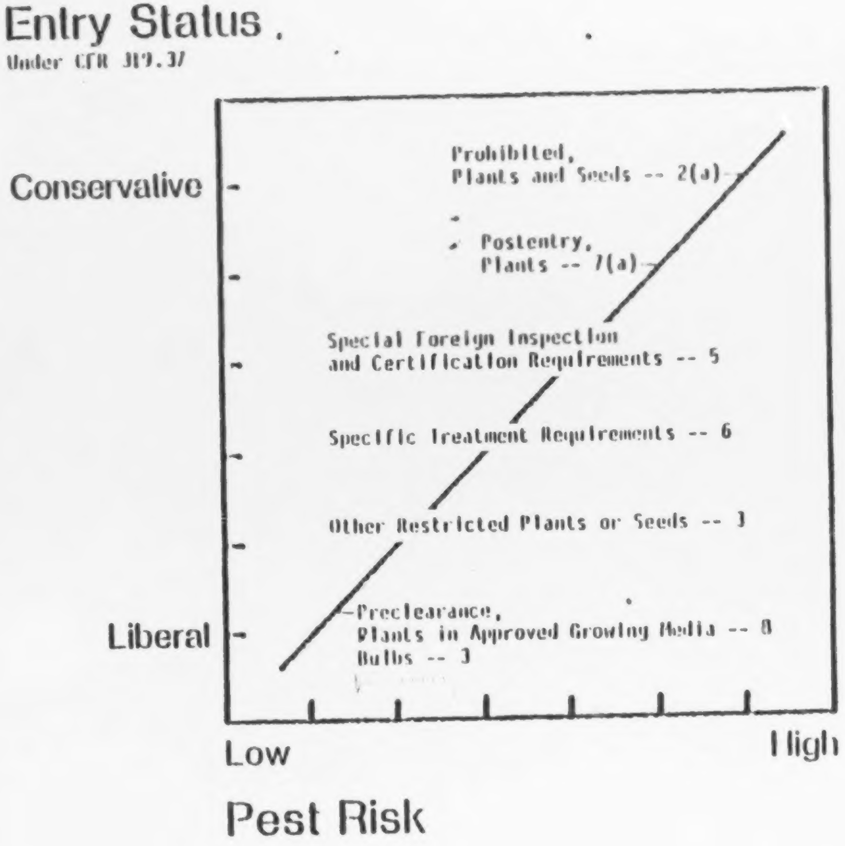


Figure 4. Rules promulgated under the Plant Quarantine Act placed on the pest risk biological curve and arranged according to a matching of pest risk and entry status. (Numbers refer to paragraphs in Code of Federal Regulations 319.37, Title 7, Chapter III).

Table 1. Number of dangerous pests that threaten U.S. in selected geographic regions.1/

GEOGRAPHIC REGION	NUMBER OF INSECTS	PATHOGENS	TOTAL PESTS
Southern Europe and Turkey	24	10	34
Northern Europe and Scandinavia	22	13	35
Russia and Eastern Europe	22	6	28
Central Africa	7	9	16
Malay and Oceania	9	12	21
India and Pakistan	3	17	20
Japan	9	12	21
United Kingdom	14	3	17
China	7	5	12
Canada	4	4	8

1/ Data extracted from: MacGregor, R., The Emigrant Pests, Animal and Plant Health Inspection Service, USDA, Washington, DC 1973

Table 2. Number of Seed-borne pathogens reported in the world scientific literature for 23 crops and the classification of these organisms into groups based on their occurrence in the United States

SCIENTIFIC NAME	COMMON NAME	TOTAL	NUMBER IN GROUP A	B	C
Arachis hypogaea	peanut	156	31	73	52
Avena sativa	oats	55	2	41	12
Brassica spp.	brassicas	49	2	36	11
Capsicum spp.	peppers	47	3	41	3
Daucus carota	carrot	37	5	30	2
Glycine max	soybean	112	9	89	14
Hordeum spp.	barley	74	9	51	14
Lactuca sativa	lettuce	30	1	27	2
Lycopersicon	tomato	46	3	35	8
Medicago sativum	alfalfa	36	3	28	5
Nicotiana tabacum	tobacco	23	6	17	0
Oryza sativa	rice	153	28	99	26
Panicum spp.	panicum	25	6	19	0
Pennisetum spp.	millet	50	9	38	3
Phaseolus spp.	beans	85	13	63	9
Pisum sativum	peas	53	5	45	3
Secale cereale	rye	32	3	28	1
Setaria spp	millet	35	9	23	3
Sorghum spp	sorghum	137	21	91	25
Triticum spp.	wheat	121	11	85	25
Vicia faba	broadbean	39	10	28	1
Vigna spp.	cowpea	116	22	77	17
Zea mays	corn	130	13	81	36
TOTALS		1,641	224	1,145	272

Seed-borne pathogens in group--

A = Pathogens of quarantine significance; i. e., not known to occur in the United States; or, if present, not widely distributed; or for which there are insufficient data to make a determination about hazard and risk.

B = present in the United States

C = storage or "quality-type organisms"

Table 3. List of pest groups as well as natural and man-made pathways along which the pests may move.

PEST GROUPS	NATURAL PATHWAYS	MAN-MADE PATHWAYS	ARTICLES OF QUARANTINE INTEREST THAT MIGHT CARRY PESTS ON MAN-MADE PATHWAYS
* insects	+ wind and storms	@+ Agricultural cargo	# packing materials, bagging
mites	+ air currents	# Nonagricultural cargo	# soil, sand, gravel
slugs	+ ocean currents	@+ mail	@+ plants and plant parts
snails	+ surface drainage	@+ baggage	# growing media microorganism cultures living insects for scientific purposes
* fungi	+ seed dispersal	@+ smuggling	+ commodities
* bacteria	root grafting	# common carriers	# containers and crates
* nematodes	+ migration of pest species	+ ships´ stores	# used vehicles and farm equipment
* parasitic plants	vectors of (microorganisms)		
* noxious weeds	+ vector (vertebrate animals)		
protozoa	self-locomotion (e.g., swarm spores)		
algae			
* viroids			
* viruses			
spiroplasmas			
mycoplasma-like organisms			

* denotes groups which contain some members which are seedborne
+ denotes direct pathways for seeds
# denotes indirect pathways for seeds
@ denotes direct pathway for vegetative propogations

Table 4. Summary of the results of indexing tests for viruses or virus-like agents in vegetative propagations of prohibited and postentry quarantine plant importations, U.S. Plant Quarantine Station, Glenn Dale, Maryland, 1968-78.

CROP OR GENERA	NUMBER OF TESTS CONDUCTED	VIRUS POSITIVE	
		NUMBER	PERCENT
From clone collections			
Saccharum (Sugarcane)	2399	46	0.02
Theobroma (Cacao)	43	0	0
TOTAL	2442	46	0.02
From all other sources[a]			
Vitis (grapvine)	409	264	65
Solanum (potato)	424	239	56
Ipomoea (sweet potato)	50	24	48
Fruit trees[b]	518	276	53
Grasses[c]	215	10	5
Miscellaneous[d]	183	69	30
Ornamentals	114	78	68
TOTAL	1913	960	50

[a] Research stations, market places, nurseries, collected in the wilds.

[b] *Citrus*, *Cydonia*, *Olea*, *Malus*, *Prunus*, and *Pyrus*.

[c] Except *Saccharum* and its relatives.

[d] *Arachis*, *Asparagus*, *Datura*, *Dioscorea*, *Theobroma*.

Table 5. Percentage of 551 cultivated and noncultivated Solanum introductions that indexed positive and negative for virus, U.S. Plant Introduction Station, Glenn Dale, Maryland, 1958-1968.

SOURCE AND SPECIES	NUMBER OF INTRODUCTIONS	VIRUS-POSITIVE	VIRUS-NEGATIVE
		Percentage	
CULTIVATED			
Solanum tuberosum	268	80	20
Other spp.	177	63	37
NONCULTIVATED			
Solanum tuberosum	19	32	68
Other spp.	87	40	60
ALL INTRODUCTIONS	551	66	34

Table 6. Summary of virus indexing tests for wild and cultivated introduction of Arachis species U.S. Plant Introduction Station, Glenn Dale, Maryland, 1968-1970.

ARACHIS SPECIES	WILD		CULTIVATED	
	Pos 1/	Neg 2/	Pos 1/	Neg 2/
benthammi	0	0	0	2
burkartii	0	2	0	0
glabrata	0	6	2	3
glabrata var. hagenbeckii	1	3	1	1
hypogaea	0	0	0	1
martii	0	0	0	1
repens	0	1	2	3
(spp.) Arachnis species	1	26	3	2
villosa	2	2	0	0
villosa var. correntina	1	1	0	0
TOTAL	5	41	8	13
PERCENTAGE	11	89	38	62

1/ Pos = indexed as positive for virus
2/ Neg = indexed as negative for virus

Table 7. The 38 most frequently prohibited genera in the quarantine regulations of 125 countries.

CROPS/GENERA	NUMBER OF COUNTRIES IN WHICH CROPS/GENERA ARE PROHIBITED	PERCENTAGES OF COUNTRIES WHICH NAME ONE OR MORE PESTS OR PATHOGENS	PERCENTAGES OF COUNTRIES PROHIBITING		
			Plants only 1/	Plants and seeds 1/	Seeds only
FOREST CROPS					
Acer (maple)	14	43	100	0	0
Castanea (chestnut)	34	23	76	24	0
Conifers 2/	27	26	100	0	0
Crataegus (hawthorn)	14	86	100	0	0
Juglans (walnut)	21	48	100	0	0
Populus (popular)	27	44	93	7	0
Quercus (oak)	25	47	92	8	0
Salix (willow)	22	45	100	0	0
Sorbus (ash)	24	58	96	4	0
Ulmus (elm)	32	47	84	16	0
FRUIT CROPS					
Citrus (citrus)	62	45	55	45	0
Cocos (coconut)	28	32	29	64	7
Fragaria (strawberry)	20	55	65	35	0
Musa (banana)	39	39	54	46	0
Pome Fruits 3/	37	68	85	15	0
Prunus (cherry, plum, etc.)	37	68	85	15	0
Ribes (current)	16	38	69	31	0
Vitis (grapevine)	41	41	90	10	0
VEGETABLE CROPS					
Ipomoea (sweetpotato)	23	35	61	39	0
Solanum (potato)	48	60	77	23	0

Table 7 (Continued)

OTHER CROPS					
Coffea (coffee)	49	31	24	57	18
Gossypium (cotton)	52	23	25	61	14
Helianthus (sunflower)	15	40	20	80	0
Hevea (rubber)	28	50	29	71	0
Nicotiana (tobacco)	26	31	35	58	7
Palm, oil	16	38	56	44	0
Rice	33	42	21	61	18
Rosa (rose)	22	41	100	0	0
Theobroma (cacao)	43	42	19	79	2
Tea	20	45	45	55	0
Saccharum (sugarcane)	40	10	63	37	0

1/ Includes plant as well as any parts for vegetative propagation.

2/ Refers to conifers as a group or specifically to Picea, Larix, Pinus, and Abies.

3/ Chaenomeles, Cydonia, Malus, and Pyrus.

Table 8. Examples of plants prohibited under the Plant Quarantine Act but which may enter as germplasm and under safeguards specified in a Departmental permit.

COMMON NAME	GENUS OR OTHER TAXA	COUNTRIES FROM WHICH PROHIBITED
Fir	*Abies* spp.	All countries except Canada
Maple	*Acer* spp.	Europe, Japan
Kiwi	*Actinidia* spp.	Japan, Taiwan
Aegilops	*Aegilops* spp.	Many countries
* Peanut	*Arachis* spp.	People's Republic of China, Thailand
Bamboo	Poaceae	All countries
Cedar	*Cedrus* spp.	Europe
Chrysanthemum	*Chrysanthemum* spp.	Many countries
Citrus	*Citrus* spp.	All countries
Coconut	*Cocos nucifera*	All countries, except seeds from Jamaica
Quince	*Cydonia* spp.	All countries
Oil palm	*Elaeis* spp.	All countries
Eucalyptus	*Eucalyptus* spp.	Argentina, Europe, Sri Lanka, Uruguay
Strawberry	*Fragaria* spp.	Australia, Northern Europe, Japan, Lebanon, New Zealand
Ash	*Fraxinus* spp.	Europe
Gladiolus	*Gladiolus* spp.	Africa plus eight countries
* Cotton	*Gossypium* spp.	All countries
Hibiscus	*Hibiscus* spp.	Africa, Brazil, India and Trinidad and Tobago
Sweetpotato	*Ipomoea* spp.	All except Canada
Larch	*Larix* spp.	Europe, Japan
Lentil	*Lens* spp.	South America
Apple	*Malus* spp.	All countries (with some postentry quarantine exceptions)
* Cassava	*Manihot esculenta*	All except Canada
* Rice	*Oryza* spp.	All countries
* Millet	*Pennisetum* spp.	Many countries
Date	*Phoenix* spp.	All countries
Spruce	*Picea* spp.	Europe, Japan, Siberia
Pine	*Pinus* spp. (2 or 3 leaved)	Europe, Japan
Grasses as plants	Poaceae	All countries except Canada
* Stone fruits	*Prunus* spp.	All countries (with some exceptions)
Pear	*Pyrus* spp.	All countries (with some exceptions)
Oak	*Quercus* spp.	Japan
Current	*Ribes nigrum*	Australia, New Zealand, Europe; British Columbia, Canada
Rose	*Rosa* spp.	Australia, Italy, New Zealand
Raspberry	*Rubus* spp.	Europe
* Sugarcane	*Saccharum* spp.	All countries
Willow	*Salix* spp.	Germany, United Kingdom, Netherlands
* Millet	*Setaria pallide-fusca*	Many countries
* Potato	*Solanum* spp.	All countries except Canada
* Sorghum	*Sorghum* spp.	Many countries
Ash	*Sorbus* spp.	Australia, China, Germany, Japan, New Zealand, Oceania, Philippine Islands
Cacao	*Theobroma* spp.	All countries
* Triticale	*Triticosecale*	Many countries

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Table 8 (Continued)

* Wheat	*Triticum* spp.	Many countries
Elm	*Ulmus* spp.	Europe
Grapevine	*Vitis* spp.	All except Canada
* Corn	*Zea mays*	Many countries
* All crops	Seed when in pulp or fruit	All countries

* including seeds

TABLE 9. Number of departmental permits issued to allow entry, under safeguards specified in the permit, of prohibited seed for experimental planting (5 crops, May 1984 - April 1985)

CROP	COUNTRIES FROM WHERE PROHIBITED	TOTAL NUMBER OF PERMITS ISSUED	COUNTRIES FROM WHICH IMPORTATION WAS AUTHORIZED	NUMBER OF PERMITS FOR EACH COUNTRY OR GROUP OF COUNTRIES
Cotton	All	4	Jamaica	1
			China	1
			India	1
			All countries	1
Rice	All	22	Thailand	1
			Philippines	9
			Colombia	1
			Peru	1
			S. Africa	1
			All other countries	9
Sorghum	Africa (all countries); Australia; Pakistan to Japan	6	India	3
			Kenya	1
			Botswana	1
			All other countries	1
Wheat	36 countries	50	Mexico	23
			Greece	1
			Israel	1
			Australia	4
			China	3
			Morocco	1
			Alfghanistan	2
			Iran	1
			Pakistan	3
			India	1
			Chile	2
			Nepal	1
			All other countries	7
Corn	Africa, eastward to Japan	9	Egypt	1
			New Zealand	2
			South Africa	1
			Five countries in Africa	2
			All countries	3

APPENDIX A

A Concept of Pest Risk Analysis

The author review (Kahn, 1979) pest risk analysis and safeguarding and applied the concept in that reference to 2 specific cases using: (1) imported citrus plants or budwood and the citrus bacterial canker disease agent, and (2) imported stone fruit plants or budwood and the agent of the little cherry disease. In general terms, this concept is as follows:

Four terms are used in discussing pest risk analysis and the entry status of imported plant germplasm, as follows:

1. Hazard--the threat posed to U.S. agriculture by the importation of plant germplasm which may harbor a pest of quarantine or economic importance.

2. Risk--the chances that the imported germplasm contains a hazardous pests.

3. Safeguard--an action taken to lower the risk (i.e., chances) that the germplasm contains a hazardous pest.

4. Favorable benefit/risk ratios. High risk plant material is often prohibited for commercial or private purposes but may be enterable for scientific purposes provided safeguards are in place to lower the pest risk. The benefits to be derived from germplasm are considered to exceed the risks when safeguards are employed.

Pest Risk and Pest Risk Analysis

A pest risk analysis is necessary in determining the entry status of articles moved by man from one geographic region to another. Since articles may either harbor hazardous organisms or serve as carriers, estimates must be made of the risk to agriculture or the environment associated with their importation.

Imported articles range from relatively low risk carriers such as oil tankers to high risk carriers such as living plants. They include, but are not limited to, the following: baggage; mailed packages, vehicles passing through border stations; used farm equipment; passengers; agricultural and nonagricultural cargos and their containers; dunnage; and packing; plants including vegetative propagations and seeds; products manufactured from plant materials; plant products imported for consumption, cultures of microorganisms, and soil.

Pest Risk

Pest risk is based on an evaluation of its biological components or variables. These may include, but are not limited to, the following:

1. Effectiveness of inspection methodology in detecting obscure pests in imported plant materials.

2. Availability and effectiveness of treatments should a pest or pathogen be found by inspection.

3. Interaction of several independent safeguards to lower risk.

4. Existence of diseases or disorders in the country of origin for which the causal agent is unknown.

5. Knowledge about the life cycle of organisms of quarantine significance in the country of origin.

6. Availability of technical backstop in the importing country should any hazardous organism be introduced. Technical backstop is a collective term for a cadre of entomologists and plant pathologists, existence of a pest and pathogen survey, a cooperating public, availability of chemicals and application equipment or effectiveness of biological control, etc. Countries lacking an adequate technical backstop are likely to be conservative in decisions about entry status.

7. The worldwide distribution of organisms of quarantine significance as compiled from a survey of the literature, correspondence and interception records from previous shipments of plant materials.

8. Information on the probability of introduction and establishment of organisms, such as the ecological range of hazardous organisms as compared with the ecological range of their hosts in the importing country, hitchhiking ability of organisms, ease of colonizations, etc.

policy, or entry status may be based on a more conservative viewpoint that that held by plant breeders or other importers.

However, eventhough the pest risk analysis suggests a conservative viewpoint that prohibits some genera, the prohibition is directed towards imports by the general and commercial public. Germplasm that is prohibited by rule may enter for scientific purposes if there are independent safeguards that may be put into play which would lower the pest risk.

The concept of safeguards has been applied to high risk germplasm genera that are prohibited. Such genera may be enterable under a special Departmental permit that prescribes safeguards and conditions of entry.

APPENDIX B

Plant Pests Specifically Mentioned in Statutes and Quarantines of the United States

Scientific Name	Common Name	Reference
Acalitus gossypii	cotton blister mite	318.47
Acarapis woodi	honey bee tracheal mite	319.76
Adoretus sinicus	Chinese rose beetle	318.13 318.82
Adoretus sp.	a chafer	318.60
Aecidium hydrangeae-paniculatea		319.37
Aecidium mori	mulberry rust	319.37
Agropyron repens	quackgrass	Federal Seed Act
Aleurocanthus spiniferus	orange spiny whitefly	318.82
Aleurocanthus woglumi	citrus blackfly	319.37
Anastrepha fraterculus	South American fruit fly	319.56
Anastrepha ludens	Mexican fruit fly	301.64 319.56
Anastrepha spp.	fruit flies	319.56
Anastrepha obliqua	West Indian fruit fly	318.58
Anastrepha suspensa	Caribbean fruit fly	318.58
Anomala sulcatula	a chafer	318.82
Apis mellifera scutellata	African honey bee	Honey Bee Act (Sec. 3(b)) 322
Apis mellifera capensis	Cape honey bee	322
Ascosphaera spp.	entomopathogens	322
Aspergillus spp.	entomopathogens	319.76
Bacillus spp.	entomopathogens	319.76
Beauveria spp.	entomopathogens	319.76
Brachycerus spp.	weevils	319.37 319.56
Cactoblastis cactorum	cactus moth	318.13 319.37
Centaurea picris	Russian knapweed	Federal Seed Act
Ceratitis capitata	Mediterranean fruit fly	Plant Quarantine Act 318.13 319.56
Cercospora batatae	a leafspot	318.82

Scientific Name	Common Name	Reference
Chilo suppressalis	Asiatic rice borer	318.13
Chrysis spp.	bee parasites	319.76
Chrysomyxa abietis	a needle rust of *Abies* spp.	319.37
Chrysomyxa himalensis	spruce needle rust	319.37
Chrysomyxa ledi var. *rhododendri*	rhododendron-spruce needle rust	319.37
Cirsium arvense	Canada thistle	Federal Seed Act
Coccus viridis	green scale	318.13
		318.82
Coelioxys spp.	bee parasites	319.76
Coniothyrium sp.		318.82
Conotrachelus spp.	weevils	319.37
Convolvulus arvensis	bindweed	Federal Seed Act
Cordyceps spp.	entomopathogens	319.76
Cronartium flaccidium	scotch pine blister rust, or resin canker	319.37
Cronartium ribicola	white-pine blister rust	Plant Quarantine Act
Cryptophlebia leucotreta	false codling moth	319.56
Curculio elephas	European chestnut weevil	319.56
Curculio nucum	a weevil	319.56
Cuscuta spp.	dodder	Federal Seed Act
Cydia splendana	nut fruit tortrix	319.56
Cydia spp.	fruit moths	319.56
Dacus cucurbitae	melon fly	318.13
		318.82
		319.56
Dacus dorsalis	oriental fruit fly	318.13
		318.82
		319.56
Dacus tryoni	Queensland fruit fly	319.56
Diaporthe mali	a leaf, branch, and fruit fungus	319.37
Dyspessa ulula	a garlic moth	319.37
		319.56
Earias vittella	a bollworm	318.82
Elsinoe australis	sweet orange scab	319.28
Elsinoe batatas	sweetpotato scab	318.82
Entomophthora spp.	entomopathogens	319.76
Entyloma oryzae	leaf smut	319.55
Erwinia salicis	watermark disease	319.37
Euphorbia esula	leafy spurge	Federal Seed Act
Euproctis chrysorrhoea	browntail moth	301.45

Scientific Name	Common Name	Reference
Dacus dorsalis	oriental fruit fly	318.13 318.82 319.56
Dacus tryoni	Queensland fruit fly	319.56
Diaporthe mali	a leaf, branch, and fruit fungus	319.37
Dyspessa ulula	a garlic moth	319.37 319.56
Earias vittella	a bollworm	318.82
Elsinoe australis	sweet orange scab	319.28
Elsinoe batatas	sweetpotato scab	318.82
Entomophthora spp.	entomopathogens	319.76
Entyloma oryzae	leaf smut	319.55
Erwinia salicis	watermark disease	319.37
Euphorbia esula	leafy spurge	Federal Seed Act
Euproctis chrysorrhoea	browntail moth	301.45
Euscepes postfasciatus	West Indian sweetpotato weevil	318.30 318.82
Euvarroa sinhai	a parasitic bee mite	319.76
Exosoma lusitanica	a chrysomelid beetle	319.56
Furcaspis oceanica	red coconut scale	318.82
Fusarium fuliginosporum	a seedling disease	319.37
Globodera pallida	a potato cyst nematode	319.37
Globodera rostochiensis	golden nematode	Golden Nematode Act 301.85 319.8 319.37
Guignardia piricola	a leaf, branch, and fruit disease	319.37
Gymnosporangium asiaticum	juniper-pear rust	319.37
Halogeton glomeratus	halogeton	Halogeton Glomeratus Control Act
Heilipus lauri	an avocado seed weevil	319.37
Hemileia vastatrix	coffee rust	319.73
Hemimene juliana		319.56
Heterodera zeae	corn cyst nematode	301.90
Holotrichia mindanaona		318.82
Hymenophysa pubescens	white top	Federal Seed Act
Hypothenemus hampei	coffee berry borer	318.82 319.73
Icerya aegyptiaca	Egyptian fluted scale	318.82

Scientific Name	Common Name	Reference
Lachnellula willkommi	European larch canker	301.91
Lampides boeticus	bean butterfly	318.13
		318.82
Lepidium draba	hoary cress	Federal Seed Act
Lepidium repens	hoary cress	Federal Seed Act
Leptocoris acuta	a scentless plant bug	318.82
Lobesia botrana	European grape vine moth	319.56
Lymantria dispar	gypsy moth	301.45
Maruca testulalis	bean pod borer	318.13
		318.58
		318.82
Melanomma glumarum	glume blotch	319.37
		319.55
Metamasius spp.	weevils	319.37
Monilinia fructigena	brown rot of fruit	319.37
Monographella albescens	leaf scald	319.37
Mononychellus tanajoa	cassava mite	319.37
Neomaskellia bergii		318.82
Omphisa anastomosalis	sweetpotato vine borer	318.30
Oospora oryzetorum	a blight	319.37
		319.55
Opogona sacchari	a sugarcane moth	319.37
Ostrinia nubilalis	European corn borer	318.82
		319.41
Pectinophora gossypiella	pink bollworm	301.52
		318.47
		319.8
		319.37
		319.56
Pectinophora scutigera	pink spotted bollworm	318.82
Peronosclerospora maydis	Java downy mildew	319.24
Peronosclerospora sacchari	sugarcane downy mildew	319.24
Pestalotia disseminata	parasitic leaf fungus	319.37
Phacidiopycnis pseudotsuga	Douglas fir canker	319.37
Phakopsora pachyrhizi	soybean rust	319.37
Phenococcus manihoti	cassava mealybug	319.37
Phialophora cinerescens	a wilt	319.37
Phyllocnistis citrella	citrus leafminer	318.82

Scientific Name	Common Name	Reference
Phyllophaga spp.	white grubs	318.60
Phyllosticta colocasiophila	a leafspot	318.82
Physoderma maydis	corn brown spot or Physoderma brown spot	318.82 319.24
Phytalus sp.		318.60
Phytophthora fragariae	red stele disease	319.37
Popillia japonica	Japanese beetle	301.40
Prays endocarpa	citrus rind borer	318.82
Pseudomonas lignicola	bacterial stain	319.37
Pseudomonas savastanoi var. fraxini	canker and dwarfing disease of ash	319.37
Puccinia graminis	black stem rust	301.38 319.37
Puccinia horiana	white rust of chrysanthemum	319.37
Puccinia mccleanii	rust	319.37
Puccinia melanocephala	sugarcane rust	319.37
Pucciniastrum actinidiae	rust	319.37
Pucciniastrum areolatum	cherry spruce rust	319.37
Rhabdoscelus obscurus	New Guinea sugarcane borer	318.82
Saccharomyces spp.	entomopathogens	319.76
Sclerophthora macrospora	crazy top (corn), downy mildew (wheat), yellow tuft (grasses)	319.55
Solenopsis spp.	imported fire ants	301.81
Sonchus arvensis	perennial sow-thistle	Federal Seed Act
Sorghum halepense	Johnsongrass	Federal Seed Act
Spodoptera littoralis	Egyptian cottonworm	318.82
Spodoptera litura	rice cutworm	318.82
Stenoma catenifer	avocado seed moth	319.37
Stereum hiugense	white rot	319.37
Sternochetus mangiferae	mango weevil	318.13 319.37
Stigmina deflectans	needlecast disease	319.37
Striga spp.	witchweed	301.80
Synchytrium endobioticum	potato wart	Plant Quarantine Act
Taphrina piri	leaf distortion fungus	319.37
Tilletia indica	karnal bunt	319.59
Trogoderma granarium	khapra beetle	319.75
Tropilaelaps clareae	a parasitic bee mite	319.76

Scientific Name	Common Name	Reference
Uredo dioscoreae-alatae	a rust	318.82
Uredo gladioli-buettneri	a rust	319.37
Urocystis agropyri	flag smut	319.8 319.59
Uromyces gladioli	a rust	319.37
Uromyces nyikensis	a rust	319.37
Uromyces transversalis	a rust	319.37
Uromyces viciae-fabae	a rust	319.37
Uromycladium tepperianum	a rust	319.37
Ustilago shiraiana	bamboo smut	319.34
Valsa mali	branch canker fungus	319.37
Varroa jacobsoni	varroa mite	319.76
Verticillium alboatrum	a wilt	319.37
Xanthomonas acernea	a leaf disease	319.37
Xanthomonas albilineans	leaf scald disease	301.87
Xanthomonas ampelina	bacterial blight	319.37
Xanthomonas campestris pv. *citri*	citrus canker	301.83 301.75 318.82 319.19 319.28
Xanthomonas manihotis	cassava bacterial blight	319.37
Xanthomonas oryzae	rice bacterial leaf blight	319.37
Xanthomonas populi	canker	319.37
Xanthomonas vasculorum	gummosis disease	301.87 318.82

Noxious Weeds

A. Aquatic Weeds

Azolla pinnata	mosquito fern, water velvet	360.200
Eichornia azurea	anchored waterhyacinth, rooted waterhyacinth	360.200
Hydrilla verticillata	hydrilla	360.200
Hygrophila polysperma	Miramar weed	360.200
Ipomoea aquatica	water-spinach, swamp morning-glory	360.200
Lagarosiphon major		360.200
Limnophila sessiliflora	ambulia	360.200
Monochoria hastata		360.200

Scientific Name	Common Name	Reference
Monochoria vaginalis		360.200
Sagittaria sagittifolia	arrowhead	360.200
Salvinia auriculata	giant salvinia	360.200
Salvinia biloba	giant salvinia	360.200
Salvinia herzogii	giant salvinia	360.200
Salvinia molesta	giant salvinia	360.200
Sparganium erectum	exotic burreed	360.200
Stratiotes aloides	water-aloe	360.200

B. Parasitic Weeds

Scientific Name	Common Name	Reference
Cuscuta spp. other than the following species: *Cuscuta americana* *Cuscuta applanata* *Cuscuta approximata* *Cuscuta attenuata* *Cuscuta boldinghii* *Cuscuta brachycalyx* *Cuscuta californica* *Cuscuta campestris* *Cuscuta cassytoides* *Cuscuta ceanothii* *Cuscuta cephalanthii* *Cuscuta compacta* *Cuscuta corylii* *Cuscuta cuspidata* *Cuscuta decipiens* *Cuscuta dentatasquamata* *Cuscuta denticulata* *Cuscuta epilinum* *Cuscuta epithymum* *Cuscuta erosa* *Cuscuta europaea* *Cuscuta exalta* *Cuscuta fasciculata* *Cuscuta glabrior*	dodders	360.200

Scientific Name	Common Name	Reference
Cuscuta globulosa		
Cuscuta glomerata		
Cuscuta gronovii		
Cuscuta harperi		
Cuscuta howelliana		
Cuscuta indecora		
Cuscuta jepsonii		
Cuscuta leptantha		
Cuscuta mitriformis		
Cuscuta nevadensis		
Cuscuta obtusiflora		
Cuscuta occidentalis		
Cuscuta odontolepis		
Cuscuta pentagona		
Cuscuta planiflora		
Cuscuta plattensis		
Cuscuta polygonorum		
Cuscuta rostrata		
Cuscuta runyonii		
Cuscuta salina		
Cuscuta sandwichiana		
Cuscuta squamata		
Cuscuta suaveolens		
Cuscuta suksdorfii		
Cuscuta tuberculata		
Cuscuta umbellata		
Cuscuta umbrosa		
Cuscuta vetchii		
Cuscuta warneri		
Orobanche spp. other than the following species:	broomrapes	360.200
Orobanche bulbosa		
Orobanche californica		
Orobanche cooperi		
Orobanche corymbosa		
Orobanche dugesii		
Orobanche fasciculata		
Orobanche ludoviciana		
Orobanche multicaulis		

Scientific Name	Common Name	Reference
Orobanche parishii		
Orobanche pinorum		
Orobanche uniflora		
Orobanche valida		
Orobanche vallicola		
Striga spp.	witchweeds	360.200

C. Terrestrial Weeds

Scientific Name	Common Name	Reference
Ageratina adenophora	crofton weed	360.200
Alternanthera sessilis	sessile joyweed	360.200
Asphodelus fistulosus	onionweed	360.200
Avena sterilis (including *Avena ludoviciana*)	animated oat, wild oat	360.200
Borreria alata		360.200
Carthamus oxyacantha	wild safflower	360.200
Chrysopogon aciculatus	pilipiliula	360.200
Commelina benghalensis	Benghal dayflower	360.200
Crupina vulgaris	common crupina	360.200
Digitaria scalarum	African couchgrass, fingergrass	360.200
Digitaria velutina	velvet fingergrass, annual conchgrass	360.200
Drymaria arenarioides	lightning weed	360.200
Emex australis	three-cornered jack	360.200
Emex spinosa	devil's thorn	360.200
Euphorbia prunifolia	painted euphorbia	360.200
Galega officinalis	goatsrue	360.200
Heracleum mantegazzianum	giant hogweed	360.200
Imperata brasiliensis	Brazilian satintail	360.200
Imperata cylindrica	cogongrass	360.200
Ipomoea triloba	little bell, aiea morning-glory	360.200
Ischaemum rugosum	murainograss	360.200
Leptochloa chinensis	Asian sprangletop	360.200
Lycium ferocissimum	African boxthorn	360.200
Melastoma malabathricum		360.200
Mikania cordata	mile-a-minute	360.200
Mikania micrantha		360.200
Mimosa invisa	giant sensitive plant	360.200
Mimosa pigra var. *pigra*	catclaw mimosa	360.200
Nassella trichotoma	serrated tussock	360.200
Opuntia auarantiaca	jointed prickly pear	360.200
Oryza longistaminata	red rice	360.200
Oryza punctata	red rice	360.200

Scientific Name	Common Name	Reference
Oryza rufipogon	red rice	360.200
Pospalum scrobiculatum	Kodo-millet	360.200
Pennisetum clandestinum	kikuyugrass	360.200
Pennisetum macrourum	African feathergrass	360.200
Pennisetum pedicellatum	kyasumagrass	360.200
Pennisetum polystachion	missiongrass, thin napiergrass	360.200
Prosopis alpataco		360.200
Prosopis argentina		360.200
Prosopis articulata		360.200
Prosopis burkartii		360.200
Prosopis caldenia		360.200
Prosopis calingastana		360.200
Prosopis campestris		360.200
Prosopis castellanosii		360.200
Prosopis denudans		360.200
Prosopis elata		360.200
Prosopis farcta		360.200
Prosopis ferox		360.200
Prosopis fiebrigii		360.200
Prosopis hassleri		360.200
Prosopis humilis		360.200
Prosopis kuntzei		360.200
Prosopis pallida		360.200
Prosopis palmeri		360.200
Prosopis reptans var. repions		360.200
Prosopis rojasiana		360.200
Prosopis ruizlealii		360.200
Prosopis ruscifolia		360.200
Prosopis sericantha		360.200
Prosopis strombulifera		360.200
Prosopis tarquata		360.200
Rottboellia exaltata	itchgrass, raoulgrass	360.200
Rubus fruticosus	wild blackberry	360.200
Rubus moluccanus	wild raspberry	360.200
Saccharum spontaneum	wild sugarcane	360.200
Salsola vermiculata	wormleaf salsola	360.200
Setaria pallide-fusca	cattail grass	360.200
Solanum torvum	turkeyberry	360.200
Tridax procumbens	coat buttons	360.200
Urochloa panicoides	liverseed grass	360.200

Names of Pests	Reference
insects of the family Bruchidae	319.37
the fruit-leaf roller complex of the family Tortricidae	319.56
andean potato latent virus	319.37
andean potato mottle virus	319.37
apple chat fruit virus	319.37
apple green crinkle virus	319.37
apple proliferation agent	319.37
apple ringspot virus	319.37
apricot chlorotic leaf roll agent	319.37
arabis mosaic virus and its strains	319.37
artichoke Italian latent virus	319.37
black currant reversion agent	319.37
cadang-cadang disease	319.37
carnation etched ring virus	319.37
carnation "fleck" virus	319.37
carnation "streak" virus	319.37
cassava African mosaic virus	319.37
cassava brown streak virus	319.37
cassava common mosaic virus	319.37
cassava latent virus	319.37
cherry leaf roll virus	319.37
cherry rusty mottle (European) agent	319.37
cotton leaf-curl virus	319.37
cotton virescence agent	319.37
Datura Colombian virus	319.37
Datura distortion or enation mosaic virus	319.37
Dulcamara mottle virus	319.37
elm mottle virus	319.37
Euonymus mosaic virus	319.37
flavescence-doree agent	319.37
grapevine fanleaf virus and its strains	319.37
grapevine leaf roll virus and its strains	319.37
grapevine vein necrosis virus	319.37
hollyhock yellow-vein mosaic virus	319.37
horsechestnut-variegation virus	319.37
Hungarian chrome mosaic virus	319.37
Jasmine-variegation virus	319.37
Kashmir virus	322
leaf chlorosis virus	319.37
lethal yellowing disease	319.37
Ligustrum mosaic virus	319.37

Names of Pests	Reference
maple-variegation virus	319.37
mountain ash variegation virus	319.37
mulberry curly little leaf agent	319.37
mulberry dwarf agent	319.37
mulberry mosaic virus	319.37
okra mosaic virus	319.37
okra yellow mosaic virus	319.37
parastolbar agent	319.37
pear blister canker virus	319.37
pear bud drop virus	319.37
plum bark split virus	319.37
plum pox (=shaika) virus	319.37
potato leaflet stunt agent	319.37
potato marginal flavescence agent	319.37
potato mop top virus	319.37
potato purple top roll agent	319.37
potato purple top wilt agent	319.37
potato spindle tuber viroid	319.37
potato virus Y(tobacco veinal necrosis strain)	319.37
potato witches broom agent	319.37
quince sooty ringspot agent	319.37
quince stunt agent	319.37
quince yellow blotch agent	319.37
raspberry ringspot virus and its strains	319.37
rice black-streaked dwarf virus	319.37
rice dwarf virus	319.37
rice grassy stunt agent	319.37
rice orange leaf agent	319.37
rice ragged stunt virus	319.37
rice stripe virus	319.37
rice transitory yellowing virus	319.37
rice tungro virus	319.37
rice yellow dwarf agent	319.37
rice yellow mottle virus	319.37
rose wilt virus	319.37
rubus stunt virus	319.37
small leaf virus	319.37
stolbar agent	319.37
strawberry latent ringspot virus and its strains	319.37
sweetpotato viruses of Eastern Africa	319.37
sweetpotato witches broom, little leaf	319.37
tobacco rattle virus	319.37
tomato blackring virus and its strains	319.37

Contract Paper No. 8

Assessment of Market Institutional Factors Affecting the Plant Breeding System

Frederick A. Bliss
University of Wisconsin

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Office of Technology Assessment
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MARKET AND INSTITUTIONAL FACTORS AFFECTING THE PLANT BREEDING SYSTEM

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Market and Institutional Factors Affecting the Plant Breeding System

Abstract

The plant breeding system begins with the assembly and evaluation of genetic resources and ends with the production of a cultivar, with the National Plant Germplasm System being an essential contributor. Changes in market and institutional factors in the public and private sectors differentially affect the various stages in the system.

Crop value and the hectarage of crop grown are the major factors influencing the number of plant breeders and programs dedicated to a crop. Other factors include the total yearly demand for new seeds, the economic feasibility of hybrid cultivars, demand for regionally-adapted cultivars, perishability of the commodity and the value per hectare.

The traditional role of public breeders in developing finished cultivars has diminished because of the perception that the private sector can assume responsibility for cultivar development in all crops and because of decreased public funding for breeding research. Viable public sector crop breeding programs should be maintained until the effects of changing roles and priorities on private-sector cultivar development can be ascertained. There is some evidence for increased cultivar development in soybeans and wheat by the private sector.

Crop (genetic) vulnerability as a real or potential national problem was brought into focus with the occurrence of the Southern corn leaf blight epidemic of 1970. Although other epidemics of this magnitude have occurred and smaller epidemics occur regularly in agriculture, the usual response is an increase in germplasm activity until the problem becomes manageable. Since the economic gains from increased productivity related to use of germplasm for breeding are diffused broadly, the costs should be borne broadly. The creation of a National Endowment for the Protection of Plant Germplasm Resources is recommended as an additional means for expanding the responsibility for germplasm preservation and use beyond just agriculture. The formation of the National Plant Genetic Resources Board in 1975 and the creation of Crop Advisory Committees were responses to the challenges of crop vulnerability. These groups are expected to play an increasing role in assuring that genetic resources problems of U.S. crop receive adequate attention.

The laws that provide proprietary rights to cultivars are controversial both from the standpoint of their desirability and their effectiveness in promoting the development of better crop cultivars. It does not appear that the Plant Variety Protection Act in itself has contributed greatly to increased genetic vulnerability, or to a concentration of breeding activities in a few companies. Increased support for graduate training of plant breeders and for crop breeding research in support of cultivar development, particularly for "minor" crops, is needed to maintain the preeminance of the U.S. plant breeding system in both the private and public sectors. The desirability of high performing hybrids and the ability to control the inbred parents biologically rather than by legal means is as potent as PVP in promoting uniformity. The creation of official lists of acceptable cultivars such as in the European system appears to be a stronger force in promoting uniformity than breeders rights legislation.

I. Plant genetic resources

Germplasm is defined narrowly as the genetic material located in the cell, and in the context of collection, conservation and breeding, it implies the plant materials that make up the genetic resources in a crop gene pool. Genetic resources can refer to all plants of a species; to populations within species; and to individual genes molecularly cloned and maintained in plasmids. "The germplasm resource base of any crop involves a diverse assemblage of materials that may be roughly grouped as follows: (1) currently useful cultivars, (2) the very sizeable reservoir of adapted but not currently utilized materials, (3) exotic and usually unadapted materials, and (4) the wild and weedy relatives" (McGuire and Qualset, 1984, p. 12).

Cultivar and variety are used interchangeably to designate a population of similar plants selected by whatever means for their utlity in an agricultural system. It is expected to possess some degree of uniformity and to "breed true" in the sense that it retains its distinctive characteristics from one generation to the next.

For commercial purposes, crops may be propagated by true seeds usually implying sexual reproduction (exceptions being seeds produced apomictically, e.g. certain cultivars of Kentucky bluegrass (Poa pratensis); or by clonal methods. The emerging DNA technologies may expand the traditional possibilities of asexual and sexual reproduction by making it possible to transfer genes across wider barriers or to clone large numbers of hybrid plants (Barton, 1982).

In general, marketing of cross- and mixed pollinating crops is done through open pollinated (freely intercrossing plants) or hybrid cultivars, the latter resulting from crossing two or more inbred (true breeding) lines. Marketing of self pollinating crops is done through pureline (inbred or true breeding) cultivars composed of only one genotype or a stable mixture of similar genotypes.

II. The plant breeding system

The plant breeding system begins with the assembly of plant genetic resources and ends with the production and distribution of a cultivar. It encompasses; (1) collection, (2) maintenance (including regeneration of stocks), (3) evaluation, (4) documentation, (5) development and (6) distribution of plant genetic resources. Evolution of primitive landrace cultivars involved domestication of wild progenitors by repeated saving of seed from the "best" plants. The commercial production of crop cultivars using modern breeding methods requires the work of plant breeders who rely on the science of plant breeding and several supporting disciplines.

1. Collection

Collection includes the physical gathering of seeds, pollen and vegetative propagules for placement in repositories (*ex situ* conservation) and/or the maintenance of living plants in their natural habitats (*in situ* conservation). The realization that genetic resources are disappearing rapidly has led to the emphasis on collection and *ex situ* conservation. *In situ* conservation has met with limited acceptance because of real or perceived physical difficulties and high costs of maintaining large diverse habitats, as well as limited incentives for study and implementation. A first step toward *in situ* conservation would be compilation of the genetic resources in U.S. forest reserves, parks, nature conservatories, reservations, wet lands etc. (Recommendation No. 7). In addition to inventories of native plants, a national inventory of important special and breeding collections should be made on a systematic basis, documented for public availability and where necessary, supported to prevent loss (Recommendation No. 6).

Important plant germplasm resides in the working collections of breeders. Some breeding and genetic collections are unique and should be retained as permanent, accessable collections, e.g., aneuploid stocks of cultivated wheat developed by USDA and state experiment station workers at the University of Missouri; maize stocks in the long term selection experiments at the University of Illinois; etc.

2. Maintenance

Ex situ collections are stored as sexual or asexual materials in repositories where conditions for maintaining viability, vitality and freedom from pests should be optimum. Each accession must be regenerated periodically, requiring careful sampling of the original collection to avoid loss of genes due to small sample size (genetic drift). If the integrity of each accession is to be maintained, intercrossing between accessions must be avoided. Many of the U.S. sites where crop collections are maintained possess inadequate facilities for regeneration of stocks. The most serious problems usually are associated with plant response to environmental factors. Many plants are of tropical origin and will not produce seeds during long day summers at temperate locations, hence the need for "tropical, low-latitude" regeneration facilities. Cross-pollinating crops pose problems of adequate isolation as well as suitable environment. The need to maintain each accession separately as opposed to maintaining genes (gene combinations) in a dynamic "gene pool" should be explored thorougly. Alternative forms of preservation, e.g. storage of pollen, cryopreservation, etc. should be used in addition to storage of seeds and vegetative propagules (Recommendation No. 11).

3. Evaluation

Evaluation is necessary to determine which accessions possess valuable traits for selection and hybridization. The type of information required and the traits to be evaluated vary. In addition to general information, allowance should be made for documentation of specialized data on specific traits for sub-sets of the total collection.

Some evaluations are made at plant introduction (P.I.) stations, and others by scientists with specialized capabilities. Evaluation of traits of particular interest to each breeder are made as part of most breeding programs. Recipients of seed from U.S. collections are asked to return evaluations to the documentation systems, but these informal requests often receive uneven compliance. Consequently valuable information often is not obtained. Evaluation must be more extensive for important traits on specific accessions, with more public and private resources becoming involved (Recommendation No. 3).

4. Documentation

Documentation includes a unique number for each accession and descriptors that include collection site, storage location and quantitative and qualitative information. Accurate classification, description and collection location is necessary to provide "passport descriptors" for each accession.

A 1977 feasibility study by the Germplasm Resources Information Project (GRIP) concluded that, "An information system exists within the plant genetic resources community of the United States, but this system lacks the organization, communication techniques, trained personnel and funding to satisfy the requirement of the NPGS community" (Anon., The National Plant Germplasm system, p. I-27). In response to these needs the Germplasm Resources Information Network (GRIN), an automated retrievable information system which is accessable through telecommunications, has been put into place. A program specialist in The Plant Genetics and Germplasm Institute (PGGI) is responsible for the operation of GRIN.

Even while the GRIN is becoming operational in 1985, the storage capacity appears to be insufficient for the data being gathered from evaluation of existing accessions, let alone the accessions to be collected and evaluated. Expansion of the GRIN facilities should be made to allow for the handling of additional data (Recommendation No. 5).

5. Development

Development of plant genetic resources is largely through plant breeding. Cultivar development may range from simple selection of superior plants from a population to production of hybrid or pure line cultivars after extensive crossing and selection. Well trained plant breeders are required in the private sector and in the public sector for fundamental research, training and cultivar development of some crops.

Recombinant DNA techniques offer promising methods for rapid genetic manipulations that cannot be done routinely by conventional techniques. The final stages of cultivar development requires testing of new lines and hybrids in areas of intended production.

6. Distribution

The U.S. seed industry commonly includes all the firms that breed, produce, condition, market and distribute seeds of all kinds

(Butler and Marion, 1983). Agricultural seeds are distributed in the U.S. through marketing channels that include sales between farmers and seed companies. For some crops, farmers choose to plant seeds they or neighbors have grown, thus bypassing the marketing system to some extent. Seed companies without R&D capabilities must rely on other sources (eg. companies, ARS or SAES's) for new cultivars.

7. The National Plant Germplasm System

Although the plant breeding system and The National Plant Germplasm System (NPGS) sometimes are thought of as being synonomous, the latter is a part of the plant breeding system. The NPGS is an interactive national effort involving federal, state and private sectors. The major working components of the U.S. system are; (1) the Plant Introduction office, (2) the "working" collections located at regional stations or with curators (for certain crops, e.g. wheat, soybeans, etc.), (3) the long-term collections located at the National Seed Storage Laboratory (NSSL), (4) the user community including scientists, educators, commercial companies, growers, consumers, etc. and (5) the information management system.

III. Roles of public, private and university sectors

The breeding and distribution of improved cultivars has been so successful that it now seems almost routine. The ability to develop even more productive cultivars is often taken for granted, and there is a tendency to de-emphasize traditional breeding activities in favor of more glamorous research. Similar to the touting of mutation breeding in the 1940's as a rapid replacement for conventional hybridization and selection, such claims are made of the new biotechnology. While mutagenesis has been useful in some cases, relatively few cultivars have been produced by mutation breeding compared to those produced during the same period by conventional methods. In light of the past experience with mutation breeding, it does not seem wise to severly limit support for traditional breeding until the new methods are on line.

The care for plant germplasm resources has been viewed largely as a function of agriculture, rather than the responsibility of society to protect and nuture a sometimes fragile natural resource. Furthermore, many individual scientists utilize a relatively few plant accessions on a sporadic basis and the contributions of these accessions to improved cultivars are often rather subtle. Even though there is a diversity of users, strong user group(s) or advocates of plant germplasm are often not available when support is crucial. As Ruttan (1972) has noted, the economic gains from increased productivity are diffused broadly, thus the costs should be borne broadly. A significant portion of increased productivity can be related to breeding of improved cultivars through the use of plant germplasm. The creation of a National Endowment for the Protection of

Plant Germplasm Resources would provide an opportunity to involve participation of a broader cross-section of society in addition to agriculture (Recommendation No. 1).

1. Private sector

Seed companies. In the U.S., privately owned seed companies are the primary distributors of seeds and plants. The involvement of private plant breeders in development is devoted primarily to production of new cultivars. There is limited long-term basic research since the return on investment is more difficult to quantify. Seed company scientists are net recipients rather than contributors at most professional meetings and in the breeding journals. Most breeders in private companies express a strong desire for continuing free interchange of ideas and plant materials, particularly with public breeders (Anon., 1984c).

Seed companies play important, but sometimes limited roles in other stages of the breeding system. Some have increased seed of accessions that repositories cannot handle. The working collections of private breeders are important components of the total genetic resources base. Although not freely accessable to the public, those collections are available if emergencies arise. Numerous accessions have been evaluated by private breeders and the resulting information stored in the public network.

Recently some private companies have assumed a greater role in plant genetic resources activities. The Pioneer Plant Breeding Forum has provided important opportunities for discussing critical issues. Pioneer Hi-Bred International Inc. has also made important contributions to genetic resources research of maize. Some seed companies provide support to universities for research and graduate training as well as internships for students. Those funds, although relatively small compared to total costs of breeding research, are important contributions.

Non-profit organizations. Non-profit organizations also support the breeding system. Notable are the past contributions of the Ford and Rockefellor Foundations in establishing IRRI, CIMMYT and other centers in the international network; and their continued support for crop improvement. Without such a commitment by those foundations it is questionable whether the improved cultivars and germplasm of major crops such as maize, rice and wheat, now available worldwide, would have been produced. The contributions of private organizations to botanical gardens, arboreta, etc. for the maintenance of ornamental plants and other plant germplasm is also important.

Producer and commodity groups provide support for breeding. Those groups may be regional in scope (e.g. the Wisconsin Carrot Growers and Processors have contributed directly to carrot research for at least 20 years) or national (e.g. the soybean growers fund research on a competitive proposal basis). Funds are directed toward either specific, or broad fundamental problems, but generally do not support collection, maintenance and documentation. Although important to the system, those funds are often short-term commitments and cannot be relied on to provide continuity which is essential for a productive breeding program.

2. Public, non-university sector

United States Department of Agriculture (USDA). The USDA has the primary responsibility for collection, maintenance and documentation, and important roles along with other groups in evaluation and development. The USDA is the appropriate agency to handle collection, maintenance and documentation of the large, multi-crop repositories since those collections serve groups throughout the U.S. and worldwide.

The question of whether plant collections are being used adequately has been raised, since some feel that plant repositories should be dynamic centers of activity that enhance the use of germplasm, rather than static museums. The missions of most U.S. respositories do not include activities that enhance use of germplasm for breeding, e.g., selection, crossing, enhancement, etc. The IR-1 Solanum (potato) collection maintained at Sturgeon Bay, Wisconsin, is an exception. It is closely associated with a research program at the University of Wisconsin and the Wisconsin Agricultural Experiment Station. The director of IR-1 has a joint USDA-University appointment and as such is involved in research and student training. Germplasm enhancement is an integral part of the IR-1 program, which interacts with state and industry interests and draws substantially on their support. That program provides a model for integrated state, federal and private programs working together in different phases of the plant breeding system. The key ingredients seem to be; 1) location of the collection in conjunction with a university, 2) funding of research and graduate student training through co-operative agreements, and 3) joint participation of federal, state and private groups.

Traditionally the USDA has played an important role in cultivar development, with cultivars being released separately or in conjunction with state agricultural experiment stations (SAES).

Recently, the stated intention of the USDA is to reduce cultivar development in those crops where private industry is capable of assuming that responsibility. This becomes more critical since the SAES's are also de-emphasizing breeding of specific crops because of decreasing importance to the state or a desire to support other research. Decisions appear to be made without adequate knowledge of the intentions of the other participants in the plant breeding system. For example, public breeding of "long-day" onions has been carried on at Michigan State University, Cornell, the University of Wisconsin, Madison and in the USDA carrot & onion program located at Madison. The program at Michigan State University has been reduced, and recently the breeder at Cornell has retired. There appears to be little correspondence to determine the need, priority and support for the remaining programs. Crop advisory committees (CAC's) should be asked to play more important roles in assessing the advisability of program changes at the state and federal levels that will affect specific crops (Recommendation No. 2).

Crops with rather small acreages or limited potential to seed companies are of particular concern. A recent survey (Brooks and Vest, 1985) showed that in 1983, about 24 F.T.E.'s were devoted to florist and nursery crops, 50 to fruits and nuts and 105 to vegetables. While the total number of programs was about the same in 1983 as in 1955-60 (400 to 425), only about 200 programs were judged by administrators "to be maintained" for the next decade, with an additional 115 "probably to be maintained". Some people feel this projected decrease is of alarming proportions and that consumers as well seed companies relying heavily on public breeding programs should be concerned. Ruttan (1982) stressed the importance of maintaining viable public-sector crop breeding programs until the effects of plant variety protection (PVP) on the performance of private-sector cultivar development can be ascertained. This is true not only for the minor crops, but also other crops, and should include other factors in addition to PVP.

Other government agencies. Other agencies play relatively minor roles in the plant breeding system. The State Department is concerned with diplomatic questions over germplasm, such as the recent F.A.O. initiative dealing with limitation by third world countries of collection and exportation of indigenous germplasm.

The Soil Conservation Service provides assistance in identifying and collecting native germplasm. The Parks and Forest Service manage large tracts of land that contain germplasm *in situ*. Important domesticated plants are maintained on Native American lands and should be inventoried, sampled and maintained (Nabhan, G., 1985).

International Agricultural Centers. The crops-oriented international agricultural centers under the auspices of the C.G.I.A.R. are important to the U.S. breeding system as well as to international efforts. Some centers have mandated responsibilities as repositories for world collections of germplasm of various crops, e.g. C.I.A.T. for *Phaseolus* beans, C.I.P. for Solanum, etc. They contain some of the accessions found in U.S. collections as well as additional ones. The international centers that maintain major repositories alleviate some of the need for the U.S. to maintain extensive duplicate collections to avoid a complete loss due to a catastrophe.

Some international centers play a valuable role in collection, maintenance, evaluation and documentation activities of crops important to the U.S. breeding system, and since U.S. breeders receive considerable breeding materials they are important to the development phase.

The International Board for Plant Genetic Resources (IBPGR) has developed during the past 10 years within the CGIAR, with its directorate being in Rome and supported by the FAO. The IBPGR has been instrumental in collecting information about plant resources that exist in many countries, developing uniform guidelines for collecting, maintenance and documentation and organizing collecting expenditures. Recently a commission has been established by FAO to, among other things, monitor the activities of the IBPGR. The debate over the IBPGR now encompasses political questions concerning ownership and rights to germplasm, inter-country exchange policies, roles of multinational corporations in crop breeding, and accessability to collections, as well as scientific issues related to germplasm activities. The location of the IBPGR, type of support and the role of the IBPGR are now topics of debate. The IBPGR has played a vital role in mobilizing resources to collect and maintain endangered germplasm that may otherwise have been lost. Despite its possible shortcomings, the loss of IBPGR would be a serious blow to germplasm activities worldwide.

3. University sector

The landgrant universities and associated SAES are the universities most heavily involved in the breeding system, and particularly in cultivar development. In most university programs the development of commercial cultivars is a necessary part of plant breeding research and graduate training. Where cultivar release is a major objective, it is usually in response to grower demands or the inability or unwillingness of private seed companies to respond to perceived needs. Cultivars produced at SAES are often released and distributed through state seed certification organizations. In some cases plant variety protection (PVP) is obtained in conjunction with seed certification, or agreements for distribution are made with private companies. Traditionally new releases of breeding materials were available to private breeders either free of charge or at a minimal cost. Recently some universities are developing procedures to obtain returns to research programs in the form of user fees or royalties because of inadequate support for traditional breeding.

Training of plant breeders is almost exclusively at landgrant universities. There is a steady or perhaps increased demand for plant breeders in the U.S. and world-wide. Although genetic engineering has been widely publicized as a replacement for plant breeding, most knowledgable scientists feel that it will complement traditional breeding. Thus the demand for plant breeders and new knowledge to support applied breeding by private companies will continue and probably expand.

4. Changes in priorities, roles and responsibilities

Traditionally, there have been strong links between the SAES's, the landgrant universities and the USDA/ARS in carrying out fundamental and mission-oriented agricultural research. In some instances, ARS programs and personnel are located at universities, and through broad-form agreements between the ARS and the University, federal researchers have had opportunities available to faculty, e.g.

supervision of graduate students and access to grant funds in addition to direct support. Almost all of the plant repositories of the the NPGS are located at or in close proximity to SAES's and universities, and operate through broad-form agreements. There is currently (1985) a move to discontinue the use of broad-form agreements and to not allow the supervision of non-federal employees (e.g. graduate students, state supported technicians, etc.) by federal employees (e.g. project leaders). The implementation of such changes would have severe negative effects on the germplasm activities within the NPGS and those federal projects where breeding research and training of plant breeding students are being done. Discontinuation of the use of broad-form agreements will allow overhead to be charged on the ARS programs at Universities, with a subsequent reduction in "working" funds of at least one-third. This "loss" is unlikely to be made-up through increased federal funds. The training of plant breeders and others in germplasm activities is likely to fall even further below levels now considered to be inadequate.

Funding. Most public funding for plant breeding research has been through formula funds, e.g. Hatch Act funds. Some funds traditionally provided for breeding are now being shifted to other activities. The research funds available through the competitive grants program of the USDA have provided only minimal additional support for plant breeding and germplasm activities. While that program is useful for increased fundamental research, it is perceived that many breeding and germplasm proposals are too applied and will not be funded. Greater support for both germplasm research and traditional breeding is needed. A "Basic Research Support Program for the National Plant Germplasm System" to facilitate research that will improve the methodologies and techniques for germplasm activities has been recommended by the NPGRB at its meeting in Washington, D.C., October 9, 1985.

Research priorities. There have been substantial changes in research priorities affecting plant breeding. Genetic engineering research is being emphasized often by replacing retiring plant breeders with molecular biologists. In private companies this research is supported by new venture capital, while in the government sector support for genetic engineering has come from some new money, e.g., competitive grants and from re-assignment of priorities. In the university sector there is a substantial re-allocation of funds and faculty positions from traditional breeding activities to molecular research.

The mechanism for establishing priorities in the plant breeding system is not well defined. Traditionally decisions are made independently by universities, U.S. agencies and private companies with minimum interaction. Two recent changes may help alleviate these problems: 1) the creation of the National Plant Genetic Resources Board to advise the Secretary of Agriculture on plant germplasm problems, and 2) initiation of Crop Advisory Committees (CAC's) to serve in advisory roles to states, federal boards, commodity groups and others on a national basis. More extensive use of the CACs by state and federal institutions should be made in setting research and training priorities (Recommendation No. 2).

Training of plant breeders. Training of plant breeders is done almost exclusively at landgrant universities. Additional support for training is needed. Hatch funds to support graduate research assistantships for breeding are shrinking. Recent increases in Hatch funds are inadequate to cover state-mandated salary increases, resulting in a reduction in research assistantships assigned to breeding and other applied disciplines. The establishment of training programs for plant breeders through training grants to landgrant universities should be implemented (Recommendation No. 8).

Cultivar development. There is a substantial shift in responsibility for cultivar development from the USDA and universities to private companies. Even for the crops where there are a large number of private sector breeders, e.g. corn, there should still be some public sector involvement in crop breeding research and cultivar or line development linked to training. While it may be argued that money used previously for cultivar development will be used for basic breeding research, it remains to be seen whether such will occur. Very little new scientific information is published by private sector plant breeders. Without active involvement of public scientists, exchange of essential new ideas will be reduced greatly. For economically minor crops, continued support for public sector research is essential. For these crops, a minimum of 2 FTE's nationally in either or both the ARS and SAES's should be supported. The FTE's could be spread among four scientists each with a .5 commitment, and the programs should receive minimum funding of $200,000 per FTE annually to support related research and student training. (Recommendation No. 9).

IV. Effects of breeders rights and intellectual property rights on the breeding system

1. Laws providing protection

The Plant Patent Act of 1930 provides for the patenting of asexually propagated crops except potato and Jeruselem artichoke (Allyn, 1934). This act is administered by the U.S. Patent Office and generally follows the conditions and requirements of the regular patent law. The Plant Variety Protection Act (PVPA) of 1970 and amended in 1980 (Anon., 1970) provides protection for sexually reproduced plants for a period of 18 years.

Industrial processes including those used in the production of plant cultivars are patentable. Some examples include a patent for the process of producing hybrid corn where restorer genes are used to restore pollen fertility of otherwise pollen sterile plants. A patent was issued for a method of producing a soft red winter wheat cultivar (Anon., 1983c). The Supreme Court upheld the patentability of a genetically engineered microorganism in Diamond v. Chakabarty (Anon., 1983b), and rejected the argument that the 1930 and 1970 acts implied the exclusion of plants and microorganisms from the basic U.S. patent law (Barton, 1982). A recent decision by the appeals board of the U.S. Patent and Trademark Office will allow genetically engineered plants, seeds and tissue cultures to be patented. Whereas under other existing legislation, protection could only be obtained for a specific cultivar, the recent decision now allows a breeder to patent generically all varieties with the described change. Presumably, advances in genetic engineering that allow more precise description of newly invented cultivars was an important factor, since previously it was thought to be impossible to create a new plant only from a written description (Sun, 1985).

2. Effects on the breeding system

Concentration of the seed industry. Butler and Marion (1983) reported that public and private breeders felt that PVP contributed to the mergers involving seed companies in the 70's. However, it was less important than other factors. They found that by 1982, nearly 50% of the PVP certificates issued were held by 14 conglomerates. Butler and Marion (1983) reported that PVP has probably resulted in slight to substantial increases in seed prices, while Perrin et al. (1983) found little evidence that PVP had produced substantial increases. Price increases from 1970 to 1980 were similar for crops receiving numerous PVP certificates (eg., soybeans) and those receiving few (corn and sorghum).

Another fear expressed was that PVP would enhance the likelihood of seed firms being purchased by companies selling other inputs (fertilizer, pesticides) that are complements for seeds. Claffey (1981) concluded from a brief review of the structure of the seed, fertilizer, and pesticide industries that although there have been mergers and acquisitions, undue concentration has not occurred.

Cultivar development. The number of PVP certificates issued since 1970 shows that greatest activity was in the self-pollinated crops with highest economic value (Table 1). Few certificates were issued for corn inbreds and none for grain sorghum, crops in which hybrid cultivars prevail. The greatest activity was in soybeans, where about 30 certificates were issued each of the last 4 years. Ownership of the 170 certificates for soybean from 1970-1980 was distributed between 30 private individuals or companies and 8 public institutions. Interestingly, no certificates for common wheat were issued in 1983 or 1984. The reason for this is unknown. During 1970-1980, of the 27 certificates issued for wheat, 17 were to private and 10 were to public concerns; for cotton, 20 went to private and 5 to public owners.

The evidence presented by Butler and Marion (1983) suggests that the impact of PVP on breeding R&D expenditures and new cultivar releases has been mixed. Established firms increased R&D in the 1960's and a significant number of companies have initiated plant breeding programs since 1970. Nine seed species; corn, soybeans, wheat, cotton, alfalfa, barley, rice, peas and beans, account for roughly 90% of the total plant breeding R&D by the commercial seed industry. These also represent about 75% of the total value of seeds sold and 70% of the total PVP certificates (Butler and Marion, 1983).

In the major vegetable crops there were relatively few certificates issued except for peas and snap beans. Also there were few different recipients (usually less than 10 per year). In bean and pea, of the certificates issued between 1970-1980, all were to private companies. This raises the question of whether there was little or no actual cultivar development for these crops at public institutions or whether the cultivars developed were released for general use without protection. It is also possible that the public breeding research on these crops is being devoted to germplasm development (enhancement) rather than production of cultivars *per se*. This situation emphasizes the need for a minimum research commitment to minor crops for breeding research on those crops where cultivar development is in the private sector and those in which there is no private work (Recommendation No. 9).

Public plant breeding. The seed industry of today is built largely upon the research base developed by public plant breeding. In only a few crops have the private sector assumed responsibility for the majority of the cultivar development (e.g. corn, possible soybeans, beans, peas) and even in those, public cultivars, inbreds and advanced breeding lines still make large contributions.

The complete cessation of public cultivar development would remove a major competing source of new cultivars for the private companies. Since public cultivars are priced close to cost, they provide an important check on prices of privately produced cultivars (Butler and Marion, 1983). Public cultivars are important sources of new cultivars for companies without breeding programs. The dominance of certain crops by protected private cultivars would limit market entry and participation by firms without breeding programs.

The flow of information and germplasm has been largely unidirectional - from public institutions to private companies. While private cultivar development may flourish for awhile (particularly in the major crops) even if public sector breeding activities are de-emphasized), the lack of new scientific information regarding breeding methods etc. will eventually reduce the ability of the private sector to efficiently produce new cultivars. All but a few of the major crops will be severly affected.

Butler and Marion (1983) found that through 1980, PVP had little apparent affect on public breeding. However, since then, it appears that increased emphasis on biotechnology at the expense of traditional breeding, combined with the ability to protect new plant forms through PVP and the U.S. Patent and Trademark Office have changed the situation. Many public breeders feel there has been a substantial reduction in the emphasis on and support for cultivar development and plant breeding research in both state and federal institutions.

3. Effects on genetic diversity

Past practices suggest that whenever high yielding, more economically rewarding cultivars become available, by whatever means, farmers will quickly adopt them in response to social and market pressures that put high priority on low cost, high quality food. As new cultivars become available and are grown widely, prevailing landraces or previously bred cultivars will lose favor and disappear unless they are conserved. It is this increased availability of higher yielding crops, rather than PVP itself that is the major threat to continued existence of genetic diversity (Ruttan, 1982). It is difficult to make conclusions regarding the impact of PVP on genetic diversity because of the lack of objective measures. With that important qualification, Butler and Marion (1983) found "there is no compelling logic nor empirical evidence that PVP has been a major factor affecting genetic diversity" (p. 104).

Cultivar protection through legislation does not appear to be more potent than other factors in promoting greater genetic uniformity. In crops where legal protection is used rarely (corn, sorghum), uniformity is as great as in those where legal protection is available. It appears that whenever proprietary rights can be maintained, there will be considerable interest in producing new cultivars which will result in a rapid turnover. However, rapid turnover also suggests rapid loss of cultivars previously considered to be superior genotypes. If this loss is deemed undesirable, the NPGS must make a greater effort to collect and conserve "current" crop cultivars because they will quickly become endangered (Recommendation No. 12). This does not suggest that less emphasis should be placed on collecting wild germplasm or endangered cultivars from other parts of the world.

The effects of PVP on exchange of genetic materials is difficult to assess. Regardless of PVP most exchange of breeding lines involves public breeders with each other or with private breeders. There is relatively little exchange between private breeders and from private to public breeders. Probably the biggest factor affecting exchange will be the number of public programs for a particular crop. As the number of public programs and the support for such decrease, so will exchange of breeding materials.

"An important source of flexibility in the U.S. legislation [eg. PVP] is that it does not preclude the marketing of seed that does not go through the registration process" (Ruttan, 1982; p. 197). The European seed control systems are often confused with plant patents or PVP. There, common catalogue requirements based on official tests establish legitimate cultivars that can be grown legally (Perrin et al., 1983; Barton, 1982). Those control systems are much stronger factors in creating uniformity and loss of genetic variability than plant legislative protection. They are highly undesirable for the U.S., and other countries interested in maintaining diversity in crop plants (Recommendation No. 14).

The importance of genetic diversity whether to society in general, plant breeders, biotechnologists, seed companies etc. cannot be over-emphasized. It is essential that the effects of many factors including market forces, political discussions, scientific findings etc. on genetic diversity be monitored.

V. Major market forces that impact on cultivar development

1. Crop value and total production

The greatest number of U.S. plant breeders work on the crops with the highest agricultural value (Table 2). In 1982 (1983 acreage was lower than usual because of the PIK program), corn had the greatest production value, followed by soybean, all hay crops, winter wheat, cotton, sorghum and potato (Table 3). The most valuable vegetable crop was tomato worth about $1.1 billion.

Although the value of corn was approximately 3 times greater than for wheats, the wheat acreage exceeded that of corn, suggesting that the acreage grown as well as value is important. However, for crops such as high return vegetables and fruits, the total acreages are not related to breeding activity. Roughly four times more acres of potatoes than tomatoes were grown but the total value of potatoes was only slightly more.

The uses made of the crop contribute to stability of demand for seed. Crops used for human food and livestock feed and are exported as well as used domestically usually sustain more breeding activity.

2. Commercialization of seed production and sales

Total demand for new seeds. The total yearly demand for seed affects new cultivar development. Total crop acreage is important but not the sole factor influencing demand. Although there is a large annual acreage of hay crops, most are seeded only every two or more years.

The utility of well-adapted regional cultivars influences breeding activity. When cultivars are widely adapted, fewer

Table 2. Number of BS, MS, and PhD scientists involved in private plant breeding on major United States crops in 1982.[a]

Major crop categories	BS	MS	PhD
Corn	201.9	100.3	155.1
Vegetables and fruits	95.7	62.3	96.4
Soybeans	41.8	15.9	35.9
Wheat	27.7	18.2	23.4
Forage legumes, mainly alfalfa	16.6	18.0	23.0
Grain sorghum	32.2	12.1	22.5
Cotton and other fiber crops	19.0	11.0	17.3
Sunflowers	12.8	13.0	15.0
Sugar beets	6.0	2.0	14.3
Forage and turf grasses	10.2	4.2	10.9
Barley, oats, rye, tritacales, millet	5.6	2.1	7.1
Rice	4.0	2.0	7.3
Flowers - ornamentals	12.5	4.5	4.5
Safflower	1.2	1.0	1.7
Tobacco	0	2.0	1.0
Totals	487.2	268.5	435.13

[a] Data are based on full-time scientist/year equivalents.

Source: Strosnider, 1984.

Table 3 Harvested area and product value of selected crops grown in the U.S.

Crop	1982		1983	
	Area harvested	Value	Area harvested	Value
	(,000 Acres)	($,000)	(,000 Acres)	($,000)
Corn(for grain)	73,030	22,039	51,537	14,064
Sorghum(for grain)	14,248	2,091	9,900	1,420
Wheat(all classes)	78,981	9,813	61,492	8,602
Rice	3,262	1,246	2,169	888
Soybeans	69,821	12,463	62,163	12,838
Dry edible beans	1,748	364	1,106	363
All hay crops	60,679	9,479	60,461	9,780
Potatoes	1,274	1,562	1,239	1,870
Cotton	9,728	3,410	7,331	2,486
Tomatoes(all)	417	1,132	--	--
Sweet corn(all)	623	336	--	--
Snap beans(processing)	204	107	--	--

Sources: Anon., 1982; Anon., 1984

cultivars are needed. However, corn growers are willing to pay higher prices for seed of single-cross hybrids that are high yielding in specific growing regions. This stimulates smaller regional seed corn companies. On the other hand, a relatively few pure line cultivars of winter wheat are grown routinely over large areas and there is less demand for regional cultivars. In general, there is likely to be greater demand for new narrowly-adapted cultivars in high return crops.

In perishable fresh market crops or when long storage of the commodity is not feasible, crop surpluses are less likely, e.g., lettuce, tomato, etc. Demand for those crop seeds will fluctuate less than in crops prone to periodic surpluses.

Farmer-grown seeds versus proprietary cultivars

Farmers may choose not to plant seed which they have grown for several reasons. Pests may be seed-borne, necessitating clean seed, e.g. snap bean seed free from halo blight. It may be advantageous to process and treat seeds to improve their performance, , e.g. onion seed is graded by weight and corn seed is often graded by size and shape. F_1 hybrid cultivars may be economically advantageous and farmers are willing to pay higher prices. The most research and development (R & D) activity in the private sector is for crops where there are large demands for F_1 hybrids. Hybrid seeds are non-durable products since they do not breed true after the first generation. They provide better economic opportunities than do non-nybrid seeds, even though the latter may be protected by the PVPA (Perrin et al., 1983). The greatest interest by new biotec companies will center on crops where farmer-grown seed can not be used routinely, e.g., F_1 hybrids or high value crops protected by PVP. It is possible that unique new processes that cause the seed to be "sterile" in the next generation may be developed, a possibility that some find alarming (Barton, 1982).

3. Seed Companies

Size and ownership. Entry into the seed business has been relatively easy. There is a recent trend toward the purchase of independent seed companies by large corporations, often having little prior experience in the seed business. Suggested reasons for this range from the general merger situation to a desire to integrate control of several crop production components into a single company (Claffey, 1981).

The acquisition of seed companies has had varied effects on breeding R & D activities. Some breeding programs have been discontinued after merger (this seems to be more likely for merged food processing companies where seed sales were not a major activity), while in other cases, R & D was expanded and new breeders hired. In the smaller seed companies, crop breeding was often the major activity, whereas in a conglomerate it is viewed as one of many activities, and is more vulnerable to change or elimination.

Support for breeding activities. Cultivar breeding is
of at least 10 years
time consuming and requires a long-term commitment/to be successful. Managers must be aware of those requirements and breeders able to project several years ahead to anticipated needs of the industry. Support is needed not only for R & D within the company but also for research in the public sector. A recent survey of seed companies showed that larger companies with breeding R & D favored emphasis on basic research by the

public sector but less emphasis on cultivar development. It was not surprising that smaller companies favored more cultivar development since they rely heavily on public releases, but they also favored emphasis on basic research (Anon., 1984c).

4. Biological Factors

Biological factors impact on crop development activities. The vulnerability to pests is important. Those crops with more pest problems that cannot be controlled with alternative methods (e.g., chemicals, rotation, etc.) receive more breeding attention, e.g. breeding for rust resistant cereal crops.

Different reproductive systems impact on how amenable crops are to breeding and selection. Improvement of biennial and perennial crops is expected to be slower than annual crops The lack of sexual reproduction hinders breeding efforts (e.g. obligate apomixis in some forage grasses, nucellar embryony in citrus crops, etc.)

5. Government Policies

In addition to providing support for crop research, other policies also influence breeding activities. The payment in kind (PIK) program in 1983 caused a reduction of 52 million acres of principle crops compared to 1982 (Anon., 1983a). There was decreased hiring of plant breeders in 1983 and reduced research spending. Breeding activities have since returned to pre-PIK levels.

Crop exports contribute to total demand for the crop, but often vary greatly. For example there is increased interest in breeding of dry edible beans when export demand creates increases in U.S. acreage. Because this is a relatively small acreage of a self-pollinated crop, interest in breeding diminishes when export markets shrink.

The number of research scientists involved in plant breeding activities in the public and private sectors is a good indicator of the relative amount of R and D support devoted to a crop nationwide. This is especially true for the major field crops. However, when combined figures for all vegetables and fruits and for all forages are presented, they provide little indication of the level of R and D on each "minor value" crop. The level of support for personnel and research in the public and private sectors must be obtained for each "minor" crop.

The principal factors determining level of plant breeding R and D are total crop hectarage, total crop value and value per seed (e.g. F_1 hybrids) vs. durable seed (pure line or open pollinated cultivars), and ability to produce proprietary commercial cultivars. These factors influence which crops receive greatest emphasis by private breeders and to a large extent public breeders. However, in the public sector, support levels are changing as cultivar development for "major crops" is de-emphasized in favor of other research activities.

For each crop, private breeders determine their goals based on improving the traits that allow cultivars to be most competitive among their market competitors and to maximize short-term profit. Crop yield is usually the trait receiving highest priority, but in vegetable and fruit fcrops and some field crops (e.g. bread wheat, malting barley) quality is also very important. Pest resistance is given priority only if susceptibility threatens the crop regularly or other control methods (e.g. chemicals) are not economic. Private breeders often use a single genetic mechanism for pest control as long as possible, with little emphasis on alternative procedures until absolutely necessary.

R and D by private breeders is primarily of a short-term nature. Utilization of unadapted germplasm for breeding in the private sector is usually minimal since it is considered long term. Most use of germplasm has been by public breeders, who develop lines and cultivars subsequently used by private companies.

VI. Changes in the agricultural industry

1. Farm and Production units

The number of farms in the U.S. has been declining steadily with a corresponding increase in the average farm size (Anon, 1984a). In 1980, farm pesticides (insecticides, fungicides, herbicides) accounted for about 3.5 percent of the operating expenses and 2.5 percent of the total production costs, expenditures for these materials having nearly tripled during the preceding 10 years, to $2.8 billion in 1980. The farm seed industry experienced a similar growth, with an estimated $3.6 billion being spent for seeds and plants in 1980 compared to $1.1 billion in 1971. Expenditures for all seeds rose 236%, the price of hybrid corn seed 142%, winter wheat 177%, soybeans 146%, winter barley 116%, spring barley 205% and sorgum 87% (Claffey, 1981).

An increased demand for seed was in part due to expansion of production areas and to more intensive production. Increased seed usage suggests a demand for more new cultivars. Duvick (1977) found that the turnover of commercial cultivars in some crops is quite pronounced as new ones are produced. As size of production units increases, the same or similar cultivars are grown on large contiguous areas. However, depending on the crop, most farmers will plant several cultivars to minimize risk of complete loss due to uniformity. Seed companies try to provide this diversity of cultivars to encourage growers to buy only their seeds.

Sometimes there is a need to grow different cultivars for varied market requirements. Different snap bean cultivars are preferred for fresh market use or canning and freezing as cut beans, french-style or whole beans. Although multiple usage creates a demand for different cultivars, it did not lead to greater genetic diversity in snap beans since most cultivars are inter-related (Anon, 1972).

2. Seed companies

There is concern over the trend toward economic concentration in the seed industry. Small companies have been acquired by other seed companies and conglomerates, often multinationals (Claffey, 1981). Several reasons for the acquisitions and mergers have been advanced, including financial and tax environments (Claffey, 1981; Barton, 1982; Perrin _et al_., 1983), strength of the dollar and the perception that food production will become increasingly important. Many small seed companies were owned by individuals or closely held partnerships and there were compelling considerations for sale of the company. The concerns over concentration and integration usually center on the questions of "undue" price enhancement of seeds; integration of cultivar development into companies selling chemicals, with the productivity of new cultivars being dependent on the chemicals; loyalty of conglomerates to agricultural issues; and the loss of genetic variability due to concentration of seed firms. There is considerable concern about whether large conglomerates will have the same interest in farmers and local agricultural issues as the regional companies have had in the past.

The rise in seed costs over the past decade was similar to increased costs of fertilizer and pesticides (Claffey, 1981), suggesting that undue price enhancement due to concentration did not occur. The fear that firms with major interests in energy and/or agricultural chemicals might have little incentive to develop cultivars which require less of their products does not appear to be the case. With few exceptions the leading pesticide and fertilizer firms have not become involved heavily in the seed industry, and seed companies controlling the large share of seed sales of major crops, e.g. corn are controlled independently (Claffey, 1981).

An important factor in the emergence of new seed companies up to this time has been the relative ease of market entry. This has kept seed prices down and maintained a diversity of companies and cultivars. In addition to the low start-up costs, entry was possible because of the availability of public cultivars. These cultivars provided seeds for small companies without the need for R&D capabilities which are long-term and costly. The decreased emphasis on production of finished cultivars by the public sector and the trend for larger companies to produce "protected" cultivars will make start-up of new seed companies more difficult and costly.

3. Emergence of biotech companies

The emergence of new biotechnology is sometimes touted as a replacement for conventional plant breeding. Although these methods have great potential, it is now being recognized that even with successful application of new techniques to crop improvement, traditional breeding activities will not be replaced and the need for natural genetic variation will continue. The new methods for genetic manipulation must be linked to development of cultivars to produce a marketable product. This has led to; 1) hiring of plant breeders to interact with molecular geneticists, 2) formation of joint ventures with traditional seed companies, and 3) acquisition of seed companies. Some seed companies have hired molecular biologists to expand their research capabilities. There appears to be some increase in conventional breeding activity as well as new biotech research in the private sector.

The formation of new biotech companies for plant improvement has been primarily as new ventures rather than by conversion of seed companies. The possibility that new biotech methods will add to undue concentration raises some concerns. "What could make this concentration issue serious is the possibility that DNA engineering will be applied to making the second generation of a seed artificially sterile. Plausibly any seed might be designed to make it biologically impossible for a farmer to reuse his crop for seed purposes. Such an "innate plant patent system" could pose enormous social costs in a concentrated industry" (Barton, 1982, p. 1072). However, a similar situation already exists with the sale of F_1 cultivars of corn, sorghum, onions, tomatoes, etc. The economic feasibility of F_1 hybrids depends on their superior performance relative to the increased cost of hybrid seed.

There is concern that the emergence of new biotech companies and development of proprietary items will lead to less exchange of plant materials and scientific information. However, there is wide variation among companies concerning policies for publication and exchange of ideas and materials. The restriction of the flow of germplasm into public repositories or among researchers would be undesirable regardless of the cause. Barton (1982, p. 1074) suggests that, "....just as publication of patents is intended to help provide a basis of knowledge for further innovation, samples of all patented plant material ought to be maintained in central repositories. This would help conserve germplasm; it could also help breeders in further research."

4. Social and economic forces

Consumers express a strong demand for abundant food, in a competitive market, and at reasonable prices (Claffey, 1981). In the U.S., the amount of individual income spent on food is substantially less than in most other countries. There are few indications that the majority of the people wish otherwise. Farmers and growers must respond to the market forces if they are to continue.

The report on genetic vulnerability by the National Academy of Sciences (Anon, 1972) concluded that the consumer, who ultimately controls the market, wants uniformity and low costs that must be met through efficiency of production. Furthermore the demands for efficiency are really demands for uniformity. When machines replace human power to increase efficiency, demand for uniformity is even greater. If a uniform product is to be harvested and provided to the consumer at low cost, the process usually begins with seeds that are uniform genetically. If consumers are willing to pay higher prices for a product and thus allow more handling by human labor rather than machines, the grower will usually adjust. For example, although pickling cucumbers are sometimes machine harvested, consumer demand is great enough to allow more costly harvesting by human labor.

VII. Impact of crop vulnerability on the breeding system

The devastating effects of the Southern corn leaf blight epidemic of 1970, in which an estimated 15% of the total U.S. corn crop was lost, led to coining of the term crop (genetic) vulnerability. Epidemics of this magnitude are not new, e.g., the Irish potato famine triggered by the destruction of potatoes by the late blight organism. Smaller, recurring epidemics also have occurred in some crops., e.g., red rust epidemics of wheat in the U.S. in 1916, 1935 and 1953.

A crop is potentially vulnerable when all or a large portion is grown in a restricted geographic area under a similar production system. Relatively large areas can be affected simultaneously by adverse conditions, e.g. the famines occurring across Africa resulting in part from lack of rainfall. As production areas become more widespread and alternative production systems are used, e.g. both rainfed and irrigated production, the potential vulnerability is reduced. Genetic vulnerability produces an exception, since regardless of variability of the production area and the systems used there is genetic uniformity of the plants being grown. Genetic vulnerability occurs because of; 1) a decrease in species diversity in crop communities, and 2) a decline in spacial and temporal species within farms and regions (Marshall, 1977).

Two major conclusions from the National Academy of Sciences report on "Genetic Vulnerability of Major Crops" were that "... (a) vulnerability stems from genetic uniformity; and (b) some American crops are on that basis highly vulnerable", (Anon, 1972; p. 286). The widely cited figures shown in Table 4 underscore those conclusions.

TABLE 4 Acreage and Farm Value of Major United States Crops and Extent to which Small Numbers of Varieties Dominate Crop Acreage (1969 figures)

Crop	Acreage (millions)	Value (millions of dollars)	Total Varieties	Major Varieties	Acreage (percent)
Bean, dry	1.4	143	25	2	60
Bean, snap	0.3	99	70	3	76
Cotton	11.2	1,200	50	3	53
Corn[a]	66.3	5,200	197[b]	6	71[c]
Millet	2.0	?		3	100
Peanut	1.4	312	15	9	95
Peas	0.4	80	50	2	96
Potato	1.4	616	82	4	72
Rice	1.8	449	14	4	65
Sorghum	16.8	795	?	?	?
Soybean	42.4	2,500	62	6	56
Sugar beet	1.4	367	16	2	42
Sweet potato	0.13	63	48	1	69
Wheat	44.3	1,800	269	9	50

[a] Corn includes seeds, forage, and silage.
[b] released public inbreds only.
[c] from Table 3, Chapter 8

Source: National Academy of Sciences, Genetic Vulnerability of Major Crops, Washington, D.C., 1972.

The major factors contributing to crop uniformity are; 1) harvest uniformity of crops due to consumers' demands for high-quality, low-priced produce, 2) the farmers' quest for the highest yielding cultivar that best fits a mechanized production system, 3) the increased demand for food worldwide (Anon., 1982a) and 4) breeding methods that entail intercrossing superior selections that are often related genetically.

1. Effects of perceived or actual vulnerability

The full impact of genetic vulnerability will not be known until after a serious epidemic occurs. Until that time only predictions and speculations are possible. Because the damage caused by the Southern corn leaf blight epidemic was quantified and highly publicized it has had a substantial impact on the plant breeding system.

Response to the Southern corn leaf blight epidemic. The catastrophic nature of the corn leaf blight epidemic and the impact of genetic vulnerability on food supply and consumer prices was realized by a great many people, perhaps for the first time. In direct response a study was initiated under the aegis of the National Research Council which resulted in the report "Genetic Vulnerability of Major Crops" (Anon., 1972).

The National Plant Genetics Resources Board (NPGRB) was appointed initially by the Secretary of Agriculture in 1975. "The task of the Board is to advise on policies related to the collection, description, maintenance, evaluation and effective utilization of the living resources represented by crop cultivars and primitive and wild forms of our Nation's crops" (Anon, 1984b, p.v.).

The need for greater in-depth expertise on specific crop problems led to the formation of small "Crop" committees that the NPGRB called upon periodically for advice. Those committees were consulted during the early stages of development of the GRIN system. The need for such committees led to the formation of Crop Advisory Committees (CAC) for different crops, with a facilitator being located in the PGGI of the USDA/ARS. The NPGRB recommended to the Secretary of Agriculture that these CAC's should serve their crop commodity groups and provide expert advice to individuals or organizations such as the NPGRB, PNGC, ARS, SAES and others on technical matters relating to plant germplasm, breedig and effective utilization. The role of and support for the CAC's should be expanded and encouraged (Recommendation No. 2).

Collection. Germplasm collection often increases; 1) in response to perceived needs for more variability, 2) because germplasm is known to be in danger of being lost, and 3) because an area thought to be particularly rich in diversity exists. There should be adequate genetic stocks in the collections, that either possess the needed variability or have yet to be evaluated, to meet the challenges of adverse events. The time between initiating a collecting expedition in response to a catastrophe and using any new found vulnerability for incorporation into a commercial cultivar is too long (i.e. 10-20 years) to effectively reduce genetic vulnerability.

Maintenance. Perceived or real genetic vulnerability of crops seems to have had little effect on maintenance methods and/or facilities for germplasm activities. Facilities throughout the NPGS are generally inadequate and improvements have been painfully slow. Siting of a facility for the National Small Grain Collection has been tied-up for several years with political as well as technical considerations. Although the NSSL, established in 1958, has long needed to be expanded, expansion is now only in the planning stage. (Recommendation No. 4). It is unlikely that the expanded NSSL will be available before 1990 at best. Response to some needs has been met. It was recognized in the 1970's that there were specialized needs for clonally propagated crops, e.g., fruits, nuts. Twelve new clonal respositories were proposed by the National Germplasm Committee as additions to the system, and there are new repositories operating at Corvallis, Oregon (Oregon State Univ.), Davis, California (University of California) and Geneva, New York (New York State Agricultural Experiment Station).

Evaluation. Germplasm evaluation activity usually shows a direct response to serious problems. Evaluation begins with currently available cultivars, followed by adapted, but out-of-date cultivars etc., until finally poorly adapted, exotic accessions are assessed. Commercial and public breeders usually respond first, then specialists, e.g. nematologists, pathologists, etc. may become involved.

The usual evaluation procedure is to screen accessions until a suitable source of resistance (or variability for some other trait) is found. The controlling genes are incorporated into adapted cultivars, often on a large scale (e.g. resistance to common mosaic virus in all snap beans cultivars except the Blue Lakes cultivars traces to one source, Corbett Refugee) (Anon., 1972). Often this leads to genetic uniformity albeit uniform resistance, and perhaps a feeling of false security. Less support is then given to evaluation and development of alternative systems of resistance or to additional sources of genetic variability for specific traits.

Documentation. An efficient data management system facilitates rapid response to problems by providing accurate information and seed of accessions quickly. Incorrect documentation provides a sense of false security and incomplete information does not allow accurate assessment of the real value of the collection. The ability to quickly search the data base for valuable lines and to provide seed samples to the user are essential. The GRIN system is now beginning to be used by scientists within the NPGS, but it appears that it will be filled to capacity within the next few years. It is inadequate for the increased activity anticipated over the next few years. The GRIN should be expanded as soon as possible (Recommendation No. 5).

Development. The time required to develop cultivars depends on the availability of genetic variability and the type of material in which it is found. The more poorly-adapted and the more distantly related the source to commercial cultivars, the longer the time needed for cultivar development.

The response to the Southern corn leaf blight epidemic was rapid because the seed companies had an alternative to the use of cytoplasmic-genic male sterility for hybrid production. De-tasseling had been used previously and stocks of elite inbreds were available. This demonstrates the necessity of alternative systems. since the development of corn inbreds with genetic resistance to SCLB would have taken considerably longer than changing to de-tassling, with subsequently greater losses.

Duvick (1977) concluded that although breeders are concerned about genetic vulnerability, there appeared to be no real trend toward increasing the number of cultivars or the genetic varability within cultivars. If anything the trend was toward fewer and more uniform cultivars.

Distribution. The distribution of new sources of genetic variability, breeding lines, and commercial cultivars is rapid. Public breeders usually provide advanced breeding lines to private breeders with few restrictions. Although the wide-spread use of a new cultivar could be limited by PVP thereby creating a "monopoly", under the provisions of that act the Secretary of Agriculture can declare the variety open to public use if necessary to insure adequate supply of food and fiber (Section 44).

VIII. Recommendations

1. Create a National Endowment for the Protection of Plant Germplasm Resources.

2. The Crop Advisory Committees should receive increased support and federal, state and private sectors should be encouraged to utilize them for evaluation of program changes that will affect the genetic resources and plant breeding systems of U.S. crops.

3. Increase evaluation of germplasm by utilizing state and private researchers as well as federal programs more fully. Establish better methods for obtaining evaluation data and placing it in the documentation system (GRIN).

4. The storage facilities for maintaining plant germplasm in the NPGS should be evaluated by a broad-based group. Recommendations as to adequacy and any need for additional facilities should be made to appropriate federal, state and private groups.

5. Encourage expanded use of the GRIN system. Documentation shsould be as flexible as possible. Expand the GRIN system immediately to provide for the anticipated needs of the system for the next 10-20 years.

6. Establish a national inventory of important special and breeding collections and when necessary provide means to prevent their losses.

7. Provide increased support for the inventory, collection and preservation of native U.S. plant genetic resources. Attention should be given particularly to those resources that can be conserved *in situ* in parks, conservatories, etc. and on Native American lands.

8. Develop a training grant program for training plant breeders at qualified land grant universities.

9. Provide a continuing minimum level of public support for breeding research in the state and federal sectors for improvement of economically minor crops. Public research and cultivar development of all crops should not be abandoned even though private companies are capable of assuming the responsibility in most major crops. Breeding research on minor crops could be supported in part in conjunction with training grant recipients.

10. Continue support for research on plant genetic engineering, but not at the expense of traditional plant breeding research and research on plant genetic resources.

11. Study and develop alternative methods of ex situ genetic conservation in addition to the usual maintenance of seeds and propagules, e.g. the advisability of creating intercrossing gene pools instead of maintaining numerous single accessions, pollen storage, cryopreservation etc.

12. Support greater effort to collect and maintain U.S. crop cultivars at the P.I. stations as well as in "permanent" storage of the NSSL. Many of the current cultivars are the obsolete cultivars of tomorrow.

13. Increase information about and accessibility to the seeds of cultivars granted PVP.

14. Seed control systems such as those that are common in Europe and which require official lists are very potent forces in promoting genetic uniformity and subsequent vulnerability. such systems should not be adopted in the U.S.

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Appendix Table 1.

Impacts of Biological, Governmental/Political and Economic Factors on the Various Stages in the Plant Breeding System.

BIOLOGICAL

Biological methods (e.g. cytoplasmic-genic male sterility) that facilitate production of commercial F_1 hybrid cultivars.

- Development - Allow the production of non-durable proprietary cultivars that must be purchased each year, e.g. hybrid corn, resulting in a high return to seed companies. Private R&D is concentrated on crops with maximum return opportunities.
- Distribution - Allow greater market control in crops where proprietary cultivars can be developed by large companies. Without cultivar (or inbred parent) development by public breeders, concentration of seed industry is easier and entry by small companies more difficult.
- Crop uniformity - Allows greater uniformity in cross-pollinated crops with F_1 hybrids than with open pollinated cultivars. Introduction of new F_1 cultivars is easier and cultivar turnover is more rapid than with open pollinated cultivars.

Genetic engineering techniques that faciliate development of proprietary cultivars.

- Evaluation - Greater need for well-defined traits, thus stimulating germplasm evaluation efforts.
- Development - Private R&D increases in crops with high return potential; minor crops receive little attention. Decrease in relative emphasis on conventional breeding may reduce overall efficiency of new cultivar development.
- Distribution - Concentration of seed industry may be increased into a fewer large companies. More proprietary cultivars developed and protected through PVP, patents and biological mechanisms.

Genetic vulnerability caused by susceptibility to biotic and abiotic factors.

- Collection - Accelerates efforts to collect more useful germplasm to meet real or perceived threats.
- Maintenance - Little apparent impact.
- Evaluation - Evaluation efforts increased until useful genetic variability found, emphasis then decreased.
- Development - Intensified activities to produce new breeding materials and cultivars. Competition to produce the first "resistant" cultivar increased.
- Distribution - Company with proprietary cultivar "resistant" to vulnerability agent gains sizeable market advantage.

GOVERNMENTAL/POLITICAL

Insufficient funding for public germplasm/breeding activities.

- Collection - Endangered germplasm lost. Genetic variability needed for advances thru conventional breeding and genetic engineering unavailable. Variability to meet genetic vulnerability inadequate.
- Maintenance - Facilities for maintaining, regenerating collections inadequate. Existing germplasm lost, newly collected materials unable to be stored, maintained, utilized.
- Evaluation - Value of existing collections not known. Need for new collections unable to be established.
- Development - Crop plant breeding and germplasm research inadequate to provide fundamental knowledge and breeding materials to private sector. Public cultivar development reduced; more difficult entry by new small companies. "Minor" crops improvement greatly diminished. Inadequate number of new plant breeders trained.

Proprietary Protection for crop cultivars.

- Development - Increased private R&D activity on major crops with large return opportunities (e.g. soybean). Increased private activity in crops where growers buy new seed annually (e.g. corn, snapbeans, canning peas). Possible decrease in cultivar development in public sector. Decreased public research in plant breeding and training of plant breeders.

Limited international exchange of germplasm.

- Collection - Collection activities reduced overall. 3rd World countries with natural germplasm resources have inadequate personnel, facilities and support for preserving and using germplasm. Much germplasm lost.
- Maintenance - Some germplasm lost if unable to be regenerated periodically.
- Evaluation - Cooperative public and private efforts diminished. Opportunities for evaluation over range of conditions reduced.
- Development - Development of new cultivars by conventional breeding and genetic engineering slowed for traits having inadequate variability in existing collections. 3rd World countries without breeding capabilities suffer with reduced exchange of breeding lines as well as unadapted germplasm.
- Distribution - Commercial seed companies unlikely to develop seed trade in countries that limit exchange. Cultivar protection may limit new cultivars being developed for 3rd World markets in countries without such laws.

Shifting responsibilities for cultivar development.

- Evaluation - Public evaluation may increase or decrease depending on availability of support from public/private sources.
- Development - Reduced emphasis on public cultivar development detrimental to "minor" crops and to a lesser extent the major crops. Fundamental breeding research and student training are concurrent with public cultivar (inbred) development. Reduced public cultivar development limits new entry of companies to the seed business and promotes greater concentration by fewer companies.

ECONOMIC

Consumer demand for high quality, low cost food.

- Evaluation - Evaluation of germplasm for useful "new" traits emphasized (eg. pollen sterility genes to promote hybrid production).
- Development - Cultivar development reflects consumer demands for uniform produce (eg. F_1 hybrids, pure line cultivars). Increased yields emphasized to allow low cost production. Genetic vulnerability may remain high as a consequence of uniformity.

Acquisition and consolidation of seed companies.

- Development - Consolidation of companies may either increase or decrease breeding R&D activities. Consolidation of several small companies stimulates new R&D activities, however consolidation of several companies with R&D causes some development to be expendable. Availability of proprietary protection of cultivars stimulates R&D. Companies with R&D in genetic engineering acquire seed companies to market newly developed cultivars resulting from genetic engineering.
- Distribution - Distribution is by fewer large companies. Entry by new companies more difficult, particularly if there are fewer public cultivars produced. Advertising and promotion greater by large companies for high value crops.

APPENDIX TABLE 2.

Creation of a National Endowment for the Protection of Plant Germplasm Resources

Awareness of germplasm resources. It must become more widely recognized that the plant germplasm resources that remain on the planet earth are a valuable natural resource that must be preserved and nutured if they are to continue to be a renewable resource. Without this realization by the layperson as well as the scientist, and without the concomitant necessary support, our plant resources are at best likely to be under-utilized and at worst become extinct or inaccessable.

Responsibility. Since the NPGS functions primarily within the agricultural research network of the U.S., germplasm activities are viewed largely as a responsibility of "agriculture", when in fact these activities and responsibilities should be and could be shared by other organizations and groups concerned with plants. Without the broader and more active involvement of "plant-concerned" groups outside of agriculture in addition to those traditionally involved in the NPGS, it is unlikely that the necessary support for activities required to effectively maintain and utilize plant germplasm resources will be possible.

Establishment of a National Endowment. A National Endowment should be established through appropriate legislation by the U.S. Government. It should provide for a Board of Governors that includes representatives from the U.S. land grant universities, non-land grant public and private colleges and universities with active botanical gardens and arboreta, the NPGS from the USDA/ARS, the U.S. Forest Service, the U.S. Soil Conservation Service, the Native American landholders, consumer advocate groups, U.S. farmer groups and naturalist societies. There should be provisions for an Executive Director and for a Scientific Advisory Board chosen on a rotating basis by the Board of Governors. The National Endowment should be provided with an initial sum of $50 million dollars from the federal government and an annual stipend of $500,000 to cover the costs of operation. The funds for activities of the Endowment would come from the income generated from the initial grant and money given to the Endowment by donors thereafter. The National Endowment should be legally and operationally separate from other components of the existing NPGS, but the two entities should function in a complementary fashion to enhance the preservation and utilization of plant germplasm resources in the U.S. and, in-so-far as possible, world-wide.

Objectives. The objectives of a National Endowment should be to foster opportunities for the preservation and utilization of plant germplasm resources in an ecologically and economically sound manner; and to monitor and chronicle the status of preservation and utilization of germplasm in the U.S. on a periodic basis.

Responsibilities. The responsibility of a National Endowment should be to: support meritorious proposals for study of the preservation and utilization of plant germplasm resources by bonafide individuals and organizations; consider and make recommendations on plans for implementing preservation and utilization activities, and solicit and accept funds for the support of these activities within the scope of the National Endowment.

APPENDIX TABLE 3.

Evaluation of Germplasm Within the NPGS

The germplasm held within the system and that which is collected in the future must be evaluated to a prescribed level if a suitable return on investment in these activities is to be expected. To accomplish suitable evaluation the following must be done:

Determine the level and type of evaluation needed for each accession or group of accessions. It is unlikely that all accessions in each collection can be evaluated for all traits of interest. That is an impossible task! All accessions need not be evaluated for all traits. Each CAC should be asked to develop a strategic plan for determining which accessions are to be evaluated for which traits; with priorities, estimated costs, schedule and appropriate researchers to carry out this plan. The GRIN system should be involved to assure that evaluation data can be entered into the system and become accessable.

Link evaluation with plans for collection. In-so-far as possible, a strategic plan for evaluation should be developed concurrently with requests for collecting expeditions. It is likely that the person(s) involved in collecting will best understand why collection is necessary and they may well be involved in subsequent evaluation. At the time of initiation, money for evaluation should be committed and the appropriate evaluator(s) contracted. Any person(s) contracted for evaluation should be expected to complete evaluation to receive full reimbursement. Evaluation and collection cannot be completely linked, since it would be undesirable to delay collection because of inability to implement evaluation plans.

Utilize a more comprehensive base of researchers and

APPENDIX TABLE 3 (CONT.).

organizations for evaluation. If adequate evaluation is to be accomplished more resources must be involved. For the most part, S.A.E.S.'s and private companies are under-utilized for the evaluation of germplasm. It seems that in general there is an attempt to provide funds for evaluation primarily to USDA/ARS program scientists and that other organizations are either discouraged or not encouraged to seek support for evaluation studies.

Reduce the reliance on ad hoc and volunteer evaluation. The use of voluntary evaluation and return of information on accessions distributed to recipients is very uneven and totally inadequate. While such information should be encouraged, this procedure should not be the primary means of obtaining evaluation information.

Increased funding for evaluation. Additional people would be interested in evaluation if adequate funding were available to non-ARS as well as ARS scientists. A clearly-defined contract with the results expected should be a basis for the evaluation specified by the CACs.

APPENDIX TABLE 4.

Evaluation of Germplasm Storage Facilities Within the NPGS.

Comprehensive evaluation. A comprehensive evaluation of the existing facilities and those likely to be needed during the next 25 years for proper storage of plant germplasm within the U.S. should be undertaken by a high-level study group, e.g. under the auspices of the National Academy of Sciences/National Research Council. The findings and recommendations of this study should be published in a useful form available to scientists, politicians and interested laypersons. It is intended that this study should provide a guideline for plans to be developed by appropriate federal, state and private organizations for meeting the storage and related activities of all the facilities comprising the NPGS. Consideration of the needs and adequacy of data storage and retrieval system, i.e. GRIN, should also be included.

While there obviously have been some prior evaluation of facilities and some plans for improvement, a comprehensive plan is not in evidence. Recommendations for extending, upgrading and building new facilities seem to be put forth on a haphazard basis, with political involvement often delaying implementation of badly needed improvements.

APPENDIX TABLE 5.

Development of Centers for Training and Research on Crop Breeding

Appropriate training of plant breeders. Training of future plant breeders to meet the needs of U.S. agriculture must be done at universities where plant breeding research is being actively conducted. Appropriate training of plant breeders must focus on courses and research in breeding rather than on general and advanced genetics, molecular genetics or recombinant DNA, with a course or two in breeding on the way to one's first job as a plant breeder. Research geneticists and plant breeders are not synonomous! Private companies hiring a plant breeder want a plant breeder, not a poor substitute who doesn't know a carrot from a fruit fly.

Establishment of a training grant program. Training of plant breeders has been done and will likely continue to be done almost exclusively at the land-grant universities. A program to provide training grants for training plant breeders at land grant universities should be established. This should be administered through an appropriate government agency with the administrator and the scientific review boards represented by plant breeders rather than geneticists and molecular biologists.

A minimum of 8-10 training grants should be awarded to appropriate universities throughout the U.S., to allow coverage of different geographic/climatic regions, crops, and private sector needs. Each training grant should provide for support for a minimum of 10 assistantships each year, with each student able to receive a maximum of 3 years support. Additional training funds to support student research should be provided.

Contract Paper No. 9

Historical Analysis of Genetic Resources Valuation

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OFFICE OF TECHNOLOGY ASSESSMENT

CONGRESS OF THE UNITED STATES

"HE SOWED, OTHERS REAPED"

The Valuation of Genetic Resources

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ABSTRACT

This paper attempts to lay out the concepts which are relevant to an understanding of the issue of genetic resource valuation. It stresses that such a valuation is a social process which has to be understood in its proper historical and international perspective.

Genetic resources appear "priceless" in the two opposite meanings of the term: they have an uncalculable social value like, say a work of art; and they have no market value i.e. no price, since once produced, they can be multiplied indefinitely at almost no cost.

Valuating genetic resources as commodities implies finding ways and methods to overcome this contradiction. It is a protracted process that that can be best examined in the case of cereals and oil and protein plants.

If these plant genetic ressources appear to have much in common with other factors of production, they also differ in one important regard: the economic process of production reproduces them with one important exception, hybrids. Hence, breeders' rights which prohibit and limit the sale of genetic information enbodied in commercial cultivars cannot prevent farmers from producing their own seed. The genetic information is a public good , and breeders' rights amount to a subsidy of seed companies to breeders. On the other hand, hybrids -that is a built-in self-destruction of genetic information enbodied in the seed- appear to be a more satisfactory method of valuation since they simultaneously create the physical market for seed and and high potential profits. There is good evidence that hybrid corn came about for this very reason In the future, most crops will become hybrid if public research does not actively breed new varieties.

However, the evolution towards hybrids is likely since the main consequence of a system of breeders' rights is to redirect public research out of breeding varieties which would compete with commercial cultivars. Public research is confined to non-proprietary work that is used by the private sector. In th long run, the private sector ultimately gains control over th method of breeding.

The contradiction between the immense social value of genetic resources in the wild and their value as an appropriable material is even greater than for genetic resources embodied in commercia varieties. A large international effort is necessary to preserve and maintain these resources. It can be done only in a climate of trust and cooperation that is unfortunately not promoted by the institutional developments under way in industrial countries --breeders' rights, hybrids or patents on micro-organisms-- which make it possible for private corporations to capture a large par of the immense social value of these resources and of the publi research effort which lead to the present biological breakthroughs.

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With the great navigators of the 15 and 16th century, men began not only to trade precious metals but also what we would now call germplasm, be it their own or other living organisms. Christopher Columbus brought back from his voyages maize and beans and a more virulent strain of syphilis while the Old World sent to the new one horses, cattle, wheat, small-pox and tuberculosis. In a way, genetic resources were becoming common property of humanity, albeit at times under an undesirable form.

With the expansion of colonial Europe, a more systematic development of genetic resources began to be established. Plant species began to be systematically studied, transferred from one part of the world, acclimated and grown according to the needs of the colonial powers. The mutiny on the Bounty broke out when the ship was bringing back to the Carribean the breadtree which was expected to be a cheap source of food for the slaves of the sugar plantations. The banana, the cocoa, the coffee, and the rubber trees, the cinchona (for quinine), the peanut, sugar cane and numerous other plants are now grown in areas were they were unknown. Botanical gardens became the turnkey in organizing the collection and the dispatching of plants from one part of the world to the other, at times with a total contempt for the countries from which these valuable genetic resources were shipped.

In Europe, the potato rapidly became a staple and, according to historians, by providing a cheap source of starch greatly helped the shift from farming to industry and the Industrial Revolution. The potato blight that struck the British Isles in 1845 and subsequent years greatly helped to populate the United States. The wheat industry in the Great Plains of the United States was, until the

50's, totally dependent upon the 1870's introduction of the "Turkey wheat" by the Mennonites and upon later systematic introductions from Russia. The soybean came to the U.S. in 1804 and its development relied on subsequent collections and introductions from China and Japan. Generally speaking, agriculture in the North American continent is, with the exception of corn and sunflower and a few other minor crops, heavily dependent upon introductions of plants and animals. "It is an imported agriculture." (Harlan, 1975)

It explains why the policy of collection, study, evaluation, and storage of genetic resources is more developed in the United States than in any other country: "As early as 1819, the Secretary of the Treasury issued a circular requesting Americans serving as consuls to send useful plant materials back to the United States. Formal plant exploration was conducted by the Office of the Patent Commissionner before 1862 when the Department of Agriculture (USDA) was created. In 1868, a section of Seed and Plant Introduction was establised in USDA; and ever since, through various name changes and reorganizations, some unit within the department has been charged with reponsibility for germ plasm assembly and maintenance." (Harlan, ibid.) In Europe, many cultivated species can be found in the wild along close relatives and are readily available to broaden the germplasm base whereas the U.S. has to search for them in its repositories or abroad.

Colonial powers had a vested interest in improving the efficiency of plant production in their colonies. At the end of the 18th century, the growing awareness of the importance of developing food production and agricultural productivity is shown by a number of facts. In economic thought, the Physiocrats built the first macro-economic model of production and distribution which was based on agriculture and considered it the only productive sector of the economy. Breeding began to be a specialized field of activity. In

France, three of the major seed companies, Vilmorin, Tezier and Clause, were founded to supply vegetable seeds. (*) The Merino breed was developed at the Bergerie Royale de Rambouillet in 1786, and again in 1799 from selection within a few hundred sheep introduced from Spain. The interest in sheep breeding in France and other parts of Europe stemmed from the demand for wool for the textile industry. In England, breeding cattle and sheep became a profitable hobby for the landed gentry. However, with animals, administrative devices such as herd-books could restrict the diffusion of valuable germplasm, which began to be a not-so-common property. The Shorthorn Herdbook, the first of its kind for cattle, was begun in 1822 in England and established in the United States in 1846. Breeding began to emerge as an activity in its own right that could be undertaken for the profits it generated and not solely for the increased efficiency it brought to agriculture. For some reason, breeding ornamental plants was largely in the hands of gifted amateurs even if tradesmen and scientists also carried it on.

The sugar beet, the only new species to be developed entirely from the wild in the last two centuries of European agriculture, has laid the pattern for modern breeding techniques as well as for the modern seed industry. It is no exaggeration to say that the seed industry as we know it proceeds from the experience gained from the sugar beet. The reasons are worthy of elaboration.

First, it was a State decision to develop the sugar beet crop. Napoleon, cut from his sources of sugar supplies by the Continental Blockade, decided to develop a domestic sugar production based on the

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($) For a fairly obvious reason: in many cases, (carrot, turnip, cabbage etc the farmer does not harvest a grain and seed production is a materially distinct operation.

not so sugar-rich sugar beet. Second, the plant naturally calls for a specialized seed production: on the one hand, it develops its tuber the first year and sets seed the second year; on the other hand, it does not breed "true", and the genetic stock easily deteriorates if it is not carefully monitored. Wild beets shed pollen everywhere and pollute the commercial breeding stock. Third, the efficiency of the sugar mills depends on a supply of beets with a high sugar content. Mills enforce the use of varieties and production techniques to achieve this goal. This means that a farmer would have to handle the physical production of seeds separately from the other farm activities, and that his maintaining of the characteristics of the variety --the reproduction of the genetic information-- requires a competence and a care that is beyond his capacity. Lastly, the mill puts pressure on farmers so that they use sound commercial seeds. Therefore, there is a market. Breeding, seed production and sales come under the control of a single authority. It is hardly surprising that modern breeding methods were discovered and implemented on the sugar beet, starting with L. de Vilmorin's description of his pedigree breeding technique in a memoir presented to the French Academy of Science in 1856.

The sugar beet is the first example in the plant kindom of genetic information becoming a commodity in itself.

With the rediscovery of Mendel's laws at the turn of the century, breeding which had been based on practical knowledge began to be a more systematic and scientific enterprise. Heredity, in the words of the American Breeders Magazine , the forerunner of the well known Journal of Heredity was a "business". However, the magazine complains that the extraordinary social efficiency of breeding does not materialize in comparable returns for the breeder. Only small returns, if any, can be privately appropriated. Indeed, in its first volume (1910), The American Breeders Magazine notes the paradox of those great breeders who died in poverty after bringing prosperity to entire areas. "Take, for instance Ephraïm Bull, who gave the world the Concord grape, now a standard variety, cultivated in thousands of vineyards, found in nearly every section where grapes will grow. He created wealth, luxury, refreshment and food for millions. His work is today precisely as valuable as it was on the day he first gave it to the world. That first mother-vine which thrives to this day at Concord, Mass., has multiplied its potency into the tens of millions of vines, unchanged, not losing an iota from any one of the many qualities which gave it its peculiar value. Ephraïm Bull died in poverty...at the age of 89 and the passers-by are informed by the epitaph on the plain slab marking Ephraïm Bull's grave that:

"HE SOWED, OTHERS REAPED"

In 1905, in France, the Société Pomologique raised for the first time the matter of a patent system for breeders. In the 20's, the Association Internationale des Selectionneurs (ASSINSEL, International Breeders Association) was set up for this very purpose. It started the long march towards the international breeders' rights system. The Paris Convention founding the Union pour la Protection des Obtentions Vegetales (U.P.O.V.) laid out the principles that national breeders rights systems had to respect and was signed by the original 6 member countries of European Economic Community. It has now expanded worldwide and covers about 20 countries, including the United States. In the meantime, in the United States, a plant patent act protecting vegetatively reproduced plants was adopted in 1930.

The question of genetic resources and of their value did not raise much controversy until the mid-seventies. A number of factors contributed to make it an issue both domestically and in the international arena:

- The 1970 corn blight in the U.S. destroyed 25% of the corn crop, mainly in the southern part of the country and raised the issue of genetic diversity of U.S. crops.

- The success of the "Green Revolution" wiped out traditional cultivars which had acquired specific adaptation to various environments and could have been a source of genetic diversity. Awareness arose that a race was engaged to collect genetic resources before they would disapear. The International Board for Plant Genetic Resource was set up in 1971 for this purpose.

- In 1972, two Stanford University scientists performed the first genetic manipulation, opening entirely new prospects of economic uses of genetic resources, be it for food and agriculture, chemicals, mining, health, pollution control and maybe computers. At this juncture, the value of genetic resources began to take a new meaning.

Living organisms are known to be able to synthesize almost any compounds from plastic material to complex molecules for the pharmaceutical industry. However, these potentially useful compounds require in most cases the cultivation of the plant or of the organism and the extraction of the product from it --a cumbersome and difficult process. For example, insulin has to be extracted from thousands of tons of pig pancreases, and morphine and its derivatives from the poppy plant. Genetic engineering offers the possibility of taking a shortcut: extracting the gene responsible for the synthesis of the compound, inserting it in a simple organism that could grow alone in a controlled environment sheltered from any competition, and extracting the compound. It follows that the problem of valuating genetic resources can be approached, not at the level of the very complex genetic resources embodied in wild or cultivated cultivars, but at the level of the simpler micro-organism in which the gene has been incorporated. This is exactly the process under way now with a system of protection that has been developed by extending the scope of the patent system to micro-organisms through court action. There is no doubt that some people are already considering the next step, that is the valuation of the gene itself. If we add that it is possible to synthetize genes, that these synthetic genes can be expressed, one may see the legal problems raised by a formal system of direct property and valuation of biological material: Who will own what? the drafter of the sequence? The person who has performed the synthese? The one who has found the right micro-organism or method to express the gene?

At any rate, these developments are opening some new perspectives. For example, ethno-botany, an discipline until now fairly academic and obscure, is insisting on the immense value of picking the brains of people in so-called primitive cultures to collect information on the properties of plants and other materials that they are using before both these plant and human cultivars are wiped out. The knowledge thus amassed would greatly facilitate the identification of potentially usefull chemicals and their eventual production through biotechnologies.

- The taking over of the the seed industry by the pharmaceutical and petro-chemical industry whether in the United States or in Europe.

- The adoption of breeders' rights laws by a number of countries according to the principles laid out by the Paris Convention created an international network through which cultivars could be traded. It also contributed to upsetting the working of the "breeding pipe-line", which spans from the collection of wild plants to the sale of commercial cultivars, since it is an implicit rule of market economies that publicly funded institutions should not compete with private ones. In the absence of breeders rights, and in the absence of economies of scale in seed production and processing, (see infra) (*) the whole "pipe-line" was publicly funded and organized. With breeders rights, the final steps fall under the unrestricted control of private enterprise and the goals of public research have to be redirected.

All this has contributed to the present acrimonious debate between the Third World countries and industrial countries. The former see that their genetic resources are destroyed and increasingly storec in the cold-storage rooms or gene banks of the latter. They hear the latter claiming how valuable these resources are and learn tha technological development gives them an increasing value. They reac that a single barley gene introduced from the middle east in the US barley crop is worth annually 50 millions dollars to the American barley farmer. And when they hear industrial countries deliver vibrant speeches about genetic resources being the "common property" of mankind, they cannot but realize that the self-contradiction of the

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(*) Practically, it means all grain and seed crops since the harvested grain is genetically the next year seed after a limited porcessing.

expression may be very significant: if genetic resources are at the same time "common" "property" (ethymologically "propius", "proper", "own" as in ownership), is it not that the expression means "let us put in common what is going to be, in fact, my property because I, alone, can value these resources?" The common property rhetoric resembles greatly the French law prohibiting rich and poor alike to sleep under bridges.

Nor is the common property idea entirely devoided of colonial overtones. In practice, it means "Let us use take these resources that are useless for you" i.e. let us have your oil since you ride on camelbacks. Therefore, it is not surprising that some more militant Third World countries restrict the outflow of their genetic resources and prefer to let them lay dormant and maybe disappear rather than let the rich industrial world benefit from them, either directly or undirectly --for instance by developping a crop in an other part of the world to undermine the competitive situation of a country which would control a large share of world supplies-- However, there are two important differences between genetic resources and other natural resources: the first ones are self-reproducible, the second are not. And moving some samples from one part of the world to the other does not exhaust the supply of the first, while mining the second does. Whether restrictions on the free circulation of genetic resources are justified or not, it shows that the present developments engage everyone on a mutually self-destructive course. The management of genetic resources as a common good could be more satisfactorily done given some institutional changes in developed countries.

Our task is here to try to clarify the relevant concepts . To do so, we shall limit ourselves to the case of grain crops, cereals and oilseeds. First they are staple. Second, they are the ones for which systems of valuation of genetic resources have been developed and this valuation is an historical development and not a fortuitous meeting of some pre-existing theoretical essences. Third, little would be gained by dealing directly with the complexities of ornamentals, tree or vegetable crops.

BREEDING AND THE CONCEPTS OF "VALUE" OF GENETIC RESOURCES

The problem of valuation of genetic resources arises from two different senses of "value" and the confusion between them. The relatively recent concern over the genetic uniformity of cultivated species and the implementation of programs to preserve a wide genetic diversity for crop species arise out of the notion of social value in the broadest sense. Much of the world food supply is provided directly or indirectly by a relatively small number of crop species -wheat, rice, maize, soybeans, potatoes, peanuts- and these species are at constant risk in every year and every locality from vagaries of wheather, diseases and pests. There is a general sense then that genetic uniformity in the important species increases the risk of catastrophic loss of food production and widespread hunger. The severe loss of the American maize crop in 1970 from blight to which all the widely used cultivars were susceptible, is the most famous recent example of the risks of genetic uniformity. While there are ways of assigning a dollar value to the human suffering and death (economists and lawyers pretend to do this out of professional necessity and ideological bias) there is really no sensible way in which the propect of widespread famine can be valued. On the other hand, we might attempt to estimate the cost of alternative methods of maintaining crop yields at "normal" levels despite the lack of genetic resistance to pests, deseases and drought, as one way to value genetic variation. A little reflection, however, shows that this approach cannot be taken seriously. To estimate the cost of research and production of, say, a pesticide against an as yet unknown pest with unknown biological characteristics seems, to say the least, problematical. Even if we used the average costs for already known pesticides, assuming no unique research or production breakthrough are required, the chief unassessed costs arises from the crop losses during the period of research and development. There is simply no way of knowing how long it would take to develop a chemical treatment for yet an unforeseen desease or insect. Moreover, as we have pointed out there is no numeraire satisfactory for the valuation of hunger, suffering and death during these intervening years of research. Alternative to genetic drought or pest resistance in wheat, say, suffer from a worse estimation problem if we consider genetic engineering approaches since there is little or no experience in his field that would allow estimates of cost and time. Mechanical alternatives are economic absurdities if we think for a moment of the problem of irrigating wheat production in Ukraine or in the American wheat belt. We must conclude then, that if value is thought of a social value, the value of preventing human hunger, there is no sensible way in which the valuation problem for genetic resources can be solved.

The second meaning of value, that is of the commodity value of agricultural products would seem at first sight a great deal more manageable for the problem of valuation of genetic resources. We can pose the counterfactual so common to economic calculation. If genetic resistance to know pathogen is not present in a crop, what is the expected loss in market value from the outbreaks of the pathogen of various degrees of severity? Or, alternatively, what is the cost of application of chemicals or other alternative pest control strategies? This valuation strategy, however is not the answer to the question posed. It does put a value on the presence (or absence) of a particular genetic trait in a crop. But it does not put a value on a genetic resource which is something quite different from the genetic composition of uniform cultivars in widespread use. It is here that serious scientific questions come into play in the valuation of genetic ressources.

By a genetic resource, we mean a genotypic property that is not currently characteristic of a crop specie in a particular region, but which is:

1) in a low frequency in the regional cultivars.

2) absent in regional cultivars but is characteristic in other locally specialized varieties.

3) preserved in a specially maintained "gene bank".

Before the genetic information can become valorized, it must be converted from its form as resource of type 1), 2), 3) to an actual characteristic of commercial cultivars. But the process of conversion has two aspects, a scientific and a commercial one, that both come into play in a contradictory manner that has given rise to the problem of genetic uniformity in the first place.

The process of mobilizing a genetic resource for the production of agricultural commodities depends upon the relationship between gene and characteristic. Some traits like plant height, most components of yield in the absence of desease, and some traits of resistance to pathogens and to extremes of climate, are influenced by large numbers of genes, scattered through the genome of the organism and acting in cumulative fashion. In a genetically variable cultivar, say an open-pollinated variety of corn, there will generally be genes at low fequency with a positive influence on a newly desired trait, and the problem for the plant breeder is to raise the frequency of the gene in the population. This is generally done by a process of recurrent selection over successive generations with a consequent enrichment in the frequency of the desired genes from generation to generation. Such process is slow and requires many years, especially if the characteristic of the crop must be mainatained at the same time. On the other hand, for such so-called polygenic traits no many large or elaborate bank of genetic resources is required. In maize, for example, it is extremely likely that there would be enough polygenic variation in a mixture of half-dozen non-commercial locally differentiated cultivars from around the world to provide a basis for selection of any trait of this type. As shown by the experiment carried on at the University of Illinois, breeding corn during 76 generations for high and low protein and fat has moved by about ten times the standard deviation of protein and fat content of the original material and the variability is not yet exhausted (Dudley, 1975).

The alternative genetic basis of desired traits, characteristic of certain pathogen resistance, flavors, colors, shape, sometimes plant height and stalk stiffness, male sterility, growth rate etc. is single genes of major effect. Such genes for novel traits are likely to be rare in the specie as a whole, but may be characteristic of an occasional cultivar or even of a wild relative. Such genes can be introduced relatively rapidly into commercial varieties by backcross technique which will preserve the desirable agronomic characters, although even in a recurrent backcross there is a certain disruption of already selected agronomic character so that 10-20 generations of backcrossing and selection might be required to restore the initial agronomic value. In contrast to polygenic traits, however, very large world-wide collections of cultivars are needed to have any hope of including the novel genes. If in addition, the needed gene is not characteristic of any local variety, but is rare everywhere, the hope of recovering it is very slim and would require immense expenditure of time and money to uncover. Certainly, no commerial breeder will attempt such a recovery. To give one example, Savitsky had to scrutinize 300.000 beet plants to find 5 monogerm plants among the open-pollinated variety Michigan Hybrid thus paving the way to the single seeded beet and doing away with the costly hand hoeing operation. Prior to this, Savitsky and Bordonos had found the property of monogermy in the 30's while working in U.S.S.R.

The importance of the commercial aspect of the valorization of genetic resources arises because genetic information has a third "value", distinct from its social value or its value as embodied in agricultural commodities for consumption. This is its value as a commodity in itself, that is its value realized in the sale of seed (or breeding stocks in animals) to the agricultural producer. It is our contention that the process of valorization of genetic information and the value of genetic resources is controlled and determined by this last aspect of value and that, in the end, it is the economics of the seed industry that will determine the effective value of genetic resources. To summarize the argument that will follow, the conditions for realizing profits in the breeding and seed industry are:

1) create the need for special attention to genetic resources because of drive for uniformity and proprietary rights;

2) erect barriers to the valorization of these genetic resources through breeding;

3) create a demand for cosmetic innovation at the expense of real innovations to enforce prorietary rights; and then

4) create value for genetic resources that is in contradiction both with the social value of the resource and with their value as potentially embodied in agricultural commodities.

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Genetic resources as they are embodied into a variety (or a strain) appear to be "priceless" in the two opposite meanings of the term:

- an immense and almost infinite social value on the one hand;

- and no market price -ie no part of the social value can be turned into private profits.

Overcoming this contradiction -that is valuating genetic resources by transforming them into commodities- is a protracted historical process which spans the 20th century and has been accelerating in the last two decades.

I. THE RELEVANT CONCEPTS

1. Breeding and the seed industry

Any bag of seeds presents two sets of characteristics which have to be carefully distinguished:

(1) The first set of characteristics --the seeds should weed-free, sized to fit the mechanical planters, free from seed born diseases, that they should germinate consistently to reach the proper plant density, that they should be made available when needed in a convenient form at a reasonable price-- is a function of the technical process of seed production and marketing and contributes to the profits to the seed producer under the heading of economies of scale.

(2) The second set pertains to the variety: its adaptation to the environment where it grows, earliness, disease resistance, drought and stress tolerance, yield potential, taste, technological

qualities, nutritive value and so on. These characteristics are the result of the breeder's work, whether public or private. They are embodied in the genetic framework of the variety and are of a strategic nature: the qualities and the shortcomings of a variety have far reaching consequences from seed bed preparation to processing and consumption.

For instance, cereal breeding during the last 30 years has taken advantage of low chemical fertilizer costs: the present amount of nitrogen used would be lethal to older varieties. The modern canning tomato is a masterpiece of mutual adaptation of the variety to the machine and of the machine to the variety. The whole development was a response to the potential threat of labour shortages at harvest time and became widely used only when the bracero program was ended by Congress in 1964.

2. The concepts

a) The specific factor of production implied in any biological process of production is a genetic information , be it a wheat variety or a strain of micro-organisms for the production of antibiotics.

b) Production in the economic sense implies a biological reproduction of the genetic information. It can be identical, statistically similar or different to various degrees. For autogamous plant, barring mutations, genetic information is identical. For a strain of micro-organisms, mutations may occur during production, but the chances that they affect the useful part of the genome are negligible. For animals, the extent to which useful genetic information is transmitted from parents to offsprings depends upon the heritability of the character under consideration. In the case of open-pollinated varieties of plants, both the gene frequency and random disorder are maintained and for production purposes, the offspring are only statistically identical to their parents.

c) Thus production implies a diffusion of the genetic information which is available to anybody and becomes a public good. This is in sharp contrast to the production of other kinds of commodities that are destroyed by their use. If the farmer buys fertilizers or oil for his tractor or electricity for his milking machine, he is obliged to return to his supplier for each new batch of product. Even capital goods like tractors must be replaced by purchase, albeit at a slower rate. Seed, however, once purchased, contains its own power of reproduction which is transferred to the farmer after an initial purchase.

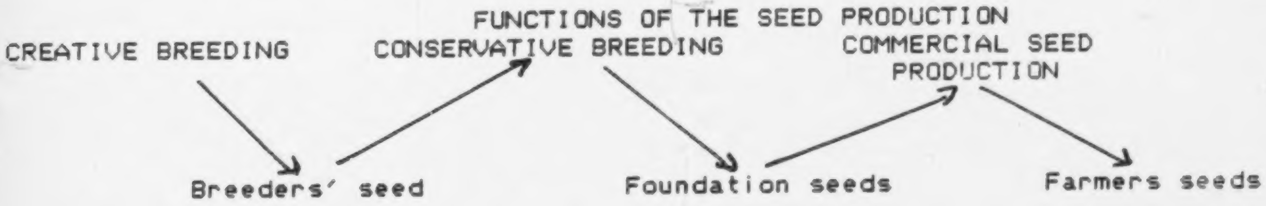
If breeders want to get any reward from their work, they must prevent the diffusion of the genetic information.

II. THE VALUATION OF GENETIC RESSOURCES EMBODIED IN COMMERCIAL VARIETIES

The organization of the seed industry

For any important grain or oil crop, seed production requires large acreage amounting to tens to hundreds of thousands acres. Seed companies do not have the land and capital to produce their seed directly and so contract farmers to produce the seed for them. Seed farmers are sold foundation or stock seeds, grow the crop under strict conditions and harvest a grain which is processed into a commercial seed. To compensate for his supplementary costs and care, the farmer under contract gets a premium over the market price of the crop if it were used for usual consumption purpose.

The production of foundation seeds is a functionally distinct operation which does not require much land. When a breeder creates a cultivar, he has a very small quantity of seed, from a few ounces to a few pounds. This small quantity has to be "increased" in order to supply seed producers with "foundation" seeds used to produce commercial seeds. We have thus two different functions: *creative breeding* on the one hand which leads to the *creation or invention* of new varieties or cultivars, and once the new cultivar has been obtained, the *maintenance* of the characteristics of the variety during the process of increasing it, that is of producing the necessary quantities of foundation seeds.


FUNCTIONS OF THE SEED PRODUCTION

In the case of corn these three functions are integrated under a single command. In the case of autogamous plants (species which "breed true", wheat, barley etc), creative and conservative breeding and, commercial seed production are done in general by distinct specialized institutions. As we shall see, the interaction of biological and economic considerations explains such different organisations.

Two systems of commercial valuation of genetic resources have been implemented:

- (1) Patents or breeders' rights to *prohibit* the sale of a cultivar without the consent of its inventor.

- (2) Hybrids to *prevent* farmers from producing their own seeds.

The first method has been implemented in Europe, particularly France since the late 20's through the regulatory activities of the State with, in 1970, a cover-up of this long standing administrative protection by a legal system of plant breeder's rights. In the United States, apart from the 1930 Plant Patent Act applying to vegetatively propagated material, it is only in 1970 that an Act of Congress (1970) created a plant breeders' rights system.

The second method has been implemented in the U.S. since 1921 through the development of F1 hybrids of maize. It is gaining ground in many species.

1. BREEDERS' RIGHTS

1.1 The origins of the European System and International law of plant breeding.

The Paris Convention creating the Union pour la Protection des Obtentions Variétales (U.P.O.V.) was signed in December 1961 by the six original members of the Common Market after 4 years of preparation by a committee of experts. To ratify the Convention, governments had to adopt Plant Protection Laws in accordance with the principles of the Convention. The way in which commercial value is created by a set of plant protection rules is most easily understood if we examine, in detail, the case of France. France has adopted such a law in 1970, the same year as the United States, which, however, was not a member of U.P.O.V. at the time.

By the beginning of the 20th century, French breeders had learned their craft, made significant scientific and technological contributions and built substantial businesses mainly on sugar beet breeding. They were frustrated when they tried to extend their trade to wheat and more generally cereal breeding, an immense market of 10 million hectares compared to the meager 300 000 ha in sugar beets. Their varieties were "pirated", sold under various names by any self-proclaimed seed producer. Ostensibly to protect farmers, an intervention of the State was required.

On the other hand, patent lawyers rejected the demand of protection on the ground that one of the basic requirements of patent law could not be achieved in the case of plant varieties: the full disclosure of the breeding work making it possible for anybody to reproduce the invention (1). Thus breeders had to create more imaginative solutions. Beginning in 1922, this was done by a serie of decrees:

a) Seeds have to be sold under their variety name, in bags mentioning the name of the variety and of the producer.

b) The breeder of the variety owns the name of the variety which is registered.
c) Only seeds of registered varieties can be offered for sale.

Therefore, the breeder owns the variety.

In 1928, a Service of "Identity and Purity" was set up within the Ministry of Agriculture to check the identity of the genetic material offered for sale and to control the purity of the seeds. Hence was "at last implemented a legal protection of new varieties which breeders have called for for such a long time." (ASSINSEL, 1931)

The catalog , originally called the register, which simultaneously sets up the right to sell a variety and the property right of the breeder, is the first pillar of the European system of protection.

The second is the certification originally introduced in Holland to protect farmers from bad potato plants. Potato plants must be produced under controlled virus-free conditions. The certification guarantees that these conditions have been met and, therefore that the plants are likely to be virus free. Fields are inspected, tests are run, and potato plants are sold with a certification tag. If the tags are numbered and mention the name of the variety and the producer of the plant, if the records are kept, then the breeder knows exactly the quantities sold.

When the French Catalog and the Dutch Certification come together, breeders find themselves protected *de-facto* (UPOV, 1974). However, there is no market mechanism to set the *price* for the genetic information. In France, an interprofessional organization of the seed trade decides each year the price of the genetic information built into the seed. This royalty represents represent 10 to 12% of the price of grain and is paid by commercial seed companies to breeders.

This system has a weakness: there is no legal basis to compel a seed company to pay the royalties. At an international level, it was a serious drawback. This is the loophole that the Paris Convention was intended to close.

Thus the legal system of protection in Europe is a cover-up for a long established administrative and regulatory practice which, far from being dismantled have kept growing. The American law is supposed to be immune from these regulatory features, but is more similar to its European counterpart than is generally acknowledged (2).

1.2 Economic consequences of breeders' rights

Annex 1 provides an analytical demonstration of the economic process determining seed prices. To understand what follows, a conceptual distinction has to be kept in mind.

On the one hand, except for hybrids, the caracteristics of a variety -the genetic information- are transmitted with little or no change from one generation to the other, be it for autogamous plants like wheat, barley and soybean (plants that "breed true") or for allogamous plants, such as corn in the times of open-pollinated varieties. In both cases, the farmer can keep a part of his harvested grain for next year seed provided he can economically process this grain into seed. The genetic information is embodied in the harvested grain and the farmer has it.

On the other hand, the harvested grain is not directly usable as seed: it contains foreign matters, weed seeds, broken or mishapen or small kernels. The seed itself may carry some deseases and may not germinate well. The harvested grain has to be processed into seed and this entails a cost. Processing can be more or less extensive and can be done more or less efficiently. An individual farmer will be fairly inefficient while a seed company will have the necessary equipment to efficiently process the grain into seed. The difference in efficiency between the two fall under the heading of economies of scale. If economies of scale are large, the farmer will rather buy his seeds from a commercial seed company. If they are narrow, he will rather make his seeds. The economic viability of a commercial seed company ultimately rests on the economies of scale in seed processing.

For cereals and oilseeds, economies of scale are usually quite limited. Processing consists of cleaning and sorting the harvested grain to get rid of foreign matter, weed seeds and broken seeds; coating the seed with fungicides and insecticides (a more difficult operation implying to handle hazardous products), and last, storing and keeping the seed in good conditions until the next growing seasong after checking that it germinates satisfactorily.

The value added by these operations is small: it represents about 30-40% of the value of the harvested grain -in the absence of coating. The sorting and cleaning equipment is cheap, easy to operate and readily available from country elevator operators at off-season time and prices. If necessary, it can be purchased and used in common, within co-ops for instance.

Out of these economies of scale, the seed company has to pay its transportation costs and the premium to contracting seed farmers and more generally all its specific expenses from overhead to taxes. In France, the premium is about 10% of the value of the grain. Whatever is left covers overhead costs, some sale and advertising expenses and of course should allow a return on investment and equity. The profitability of seed companies is thus in most cases narrow.

What happens when a breeder's right system comes into being? It gives the breeder of variety the right to collect a royalty. The royalty is paid by the seed company on the assumption that it can pass it on to the farmer . The seed company is supposed to add to all its costs --procuring the grain from contracting farmers, processing it into seed, selling it-- the royalty paid to the breeder and then to add overhead and a normal profit to reach its selling price.

However this assumes that the farmer pays for the genetic information provided by the breeder, i.e. pays for something he already has since it is embodied in his harvested grain. This is unlikely. What a farmer is willing to buy from a commercial seed company is the processing service if the seed company can provide it more efficiently than he does. Then, if farmers do not pay for the genetic information, the royalty R remains stuck with the seed company which has to pay it out of its margin in the same manner as its other specific costs such as overhead, taxes or transportation. Farmers will not pay for breeder's work so seed companies must, with a corresponding drastic decline of their profitability (3). Breeder's rights amount to a subsidy from seed companies to breeders. En England, after a breeders' right law was adopted in 1964, smaller seed companies disappeared.

A short anecdote may help to understand the argument. For a flat fee, seed companies in France used to process into seed the grain that farmers were bringing in. At first sight, such a behavior is uncomprehensible since the seed company appears to undermine its market for commercial seed. However, a closer examination shows that so doing is much more profitable than producing and selling commercial seed: the seed company does not have to pay a royalty to the breeder. It keeps the entire fee -the economies of scale- for itself while in the case of commercial seed, it has to give the breeder his royalty! For years, breeders have attempted to close this loophole and have recently succeeded.

If seed companies deem the royalty rate too high, they may consider going into breeding and selling their own varieties. R the royalty will be replaced by R' the unit cost of research and development of new varieties. Again, this solution may be less costly than paying out royalties, but these research expenses are not (for a seed codmpany) retrievable through market mechanisms. For a seed company, the best solution is that the breeding work be performed by public institutions or that varieties be a public good -a solution which is compatible with the existence of a private breeding activity through for instance a cost-plus system of contracting research work as done in many industrial sectors.

To summarize our discussion, in the absence of breeder's rights and of economies of scale in seed production, no private money will be invested in breeding except as a hobby and breeding has to be done by public institutions.

If breeder's rights are in force, some private money flows into breeding. Breeding which is profitable and seed production which is not, will ultimately be separated. But even in this case, breeding cannot be very profitable -at least by the standards of multinational corporations: if breeders increase the royalty, seed companies will go bankrupt if they don't pass it on to farmers and if they try to do so, the farmers will quickly find the price too high and revert to produce their own seed! The market for commercial seeds will shrink.

Theoretically, the matter of breeders rights comes to the following:

- breeding benefits the entire society. Should seed companies pay for it?

- if the answer is yes, will royalties will be sufficient to insure an adequate funding of such a crucial activity at a time when its scope is changing from a small scale enterprise of talented farmers to a systematic scientific and technological activity? We think it doubtful.

The above argument rests on a single assumption: that farmers are cost conscious entrepreneurs and pick up the less expensive alternative. One may well object that this is not the case and stress that marketing, advertising, do create a preference for commercial seed. It is also possible to multiply the obstacles to prevent a farmer from producing or selling his seed and the above anecdote is an example of the first. In the United States, farmers can produce their own seed but cannot advertise it for sale, a clever obstacle to increase the economies of scale and an example of the second. Indeed, varietal obsolescence through cosmetic changes of commercial varieties widen the market for commercial seed since when a variety is first released, a farmer can only get it from a seed company. The present proliferation of varieties is an ample testimony of such a policy. (*)

These arguments focuss on the wrong side of the issue. To make an analogy, they are tantamount to negate the laws of falling bodies because of friction. Friction is obviously an important phenomenon, but understanding it requires first a theory of falling bodies. Understanding the very forms of marketing in the non-hybrid seed industry requires the conceptual framework that we have laid out. Dealing with "friction", i.e. studying the different techniques, methods or institutional arrangements implemented in different countries at different times for different species to overcome the central contradiction of the non-hybrid seed industry --the "pricelessness" of genetic information-- is another assignement.

2. HYBRIDS

2.1. A alternative view of hybrid corn

F1 hybrids are not simply a way to "improve" corn more efficiently than by other methods but above all a way of creating proprietary rights on the result of breeding work.

Barton (1982) has inadvertently made this point in his review

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(*) But most farmers try the new variety on a small acreage and if they like it, can and in many cases process what they have harvested into seed for the next year!

article of breeders rights. He envisages the science fiction possibility that "D.N.A. engineering will be applied to make second generation of a seed artificially sterile... Such an 'innate patent system' could pose enormous social costs in a concentrated industry." Althought the blatant use of biological progress to make second generation seed artificially sterile would be socially unacceptable, it has not been realized that at least for 60 years hybrids have done exactly that! But the acceptance of hybrids was possible because they were claimed to "increase yields".

The F2 generation of hybrid corn, if not biologically sterile, is economically worthless as seed since it will produce anywhere from 20 to 40% less than the F1 hybrid. For all practical purposes, such a yield loss amounts to a biological sterility.

There is good scientific and historical evidence that the hybrid method of breeding has not come about because of its intrinsic superiority over alternatives, but because it opened up a new highly profitable field, breeding and seed production to private enterprise.

Without entering into details, a close examination of the evolution of yields in the United States would show that "conservative estimates of 25% to 50% yield increase due to hybrid corn" (Juggenheimer, 1976) are imaginary. In fact, Henry Wallace (1938) himself has indirectly set the record straight: he was fairly "certain that in 1945, there will be well adapted commercial strains of hybrid corn for practically every section of the Corn Belt, that will yield at least 20% more that the average open-pollinated corn as grown in the respective part in 1920." His expectations were correct as to the availability of hybrid varieties but optimistic as to yield increases: 16% in the corn belt between 1920 and 1945. Meantime, wheat yields in the US had increased 36%!

The feeding value of these hybrids was 3% below open-pollinated varieties (Sprague, 1955). The protein content of the hybrid varieties had decreased (Hayes, 1963). Hence, the 16 % yield increase is set back to 13% in nutritional value -at best. If we add that the use of commercial fertilizer in corn belt corn increased several times between the mid 30's and the mid 40's, that machinery had made fantastic strides in 25 years, that soybean acreage was increasing rapidly, providing a balanced rotation and that these factors contributed more to corn yield increase than to wheat yield increases, it casts some doubt over the intrinsic superiority of the new method of breeding. One may add that corn yields have increased 10 times faster from the 50's to the present time than during the period 1921-1945 of implementation and adoption of the hybrid breeding technique. In other terms, corn yields began to pick up after hybrids had become established, when it was at last realized that the improvement of source populations was the only method to secure yield increases in hybrids and when the impetus of public research shifted to what can be essentially considered a non-hybrid method of improvement -even if it ended up with inbred-hybrid commercial varieties.

From a scientific point of view, the soundness of the inbred-hybrid breeding method rests upon the assumption of a special, quasi-"mystical" (Eberhart, 1979) phenomenon, heterosis. Heterosis postulates that a Mendelian explanation of hybrid vigor in terms of dominance and recessiveness -which leads to a non-hybrid method of breeding- is inadequate, and that there is some supplemental "stimulation" of heterozygosity per se. Here again a careful study of the scientific litterature reveals that:

1. the postulation of heterosis as an apparent meaningful

scientific concept results from the willingness to make hybrids a commercial success, while the common view holds that hybrid corn is the result of heterosis as a real biological phenomenon (Berlan, 1985).

2. the quest to demonstrate the existence of heterosis (understood in its original sense of overdominance) has failed again and again, be it at the level of genes (Lewontin, 1973) or at the level of the corn plant (Moll and alii, 1964).

If then, the conventional rationale for the inbred-hybrid method of breeding is not a good one, what can be a reasonable explanation?

2.2 The valuation of breeding work through hybrids

Annex 2 provides an analytical demonstration of what follows. But the core of the argument is easy to understand.

With hybrids, the farmer cannot produce his own seed by picking well-shaped, desease-free ears in his field and saving them for next year seed. Or if he does, his yield will be lower by 20 to 40% in comparison of the yield of commercial hybrid seed.

Therefore, hybrids create the physical market for seed. In the early 30's, a few % of the corn crop was sown with commercial seeds: some farmers wanted to try a new open-pollinated variety that had proven successfull in his neighbour's fields, or simply, at planting time, realized that the seed they had saved from last year crop did not germinate or had been eaten by rodents. With hybrids, 100% of the corn crop was seeded with commercial seed.

This fact was widely recognized from the beginning and was considered to be the price of progress. But, this is only a part of the story and the other has not really been told. What is exactly the price of progress?

The crop loss in the case of use of farmers' seed as opposed to commercial seed is a clue to the answer. This crop loss is equal to the % of yield loss times the yield. The value to a farmer of a bushel of commercial seed corn is equal to this crop loss times the number of acres sown with a bushel of seed. If we take corn yield at 150 bu/acre, the percentage loss at 30% and the number of acres sown with a bushel of seed corn equal to 4, then the maximum price for a bushel of seed corn is 150 x 30% x 4 = 180 times the price of a bushel of corn for grain. If instead of hybrids, open-pollinated seed corn were sold, its price would be at most twice or maybe three times the price of corn for grain --the ratio that prevails for wheat seed for example-- In other terms, with hybrids, the seed price can be totally disconnected from the value of grain for consumption, the genetic information is the exclusive property of the breeder, and not the common property of all. With hybrids, the farmer is no longer the main competitor of his supplier and the regular game of inter-enterprise competition is reestablished .

Then, what are the economics of hybrid seed prices if prices can flutuate between actual costs of production --at present at most 4 to 5 times the price of corn for grain-- and almost 200 times this price?

Market structure then determines the profitability of the seed industry. If entry into the market is easy and barriers to entry low, competition and particularly price competition will be intense, actual margin and profits will be low. On the other hand, if a seed company achieves a monopoly, it can increase the price until the cost of hybrid seed offsets all the gains of using them. At a higher price, farmers would shift to their own grain for seed.

Market structure

The hybrid seed industry has quickly built up barriers to entry that guaranteed a high margin and high profits thanks to sale effort (marketing, advertising etc) and research and development.

Research and development are in fact, the key to a strategy of building barriers to entry. Not only the marketing staff and salesmen need "new" varieties to launch their compaign with, but the creation of new varieties depends on the past investments in research and development. A breeding program may take anything up to 10 years to yield a commercial variety. Seed corn companies devote 3 to 5% of their total income to their breeding activities. It may not seem a very impressive figure when compared to their sale expenses (20% or more of their total income) but when cumulated over the years, it is a formidable deterrent to entry.

III SYSTEMS OF VALUATION AND THEIR CONSEQUENCES

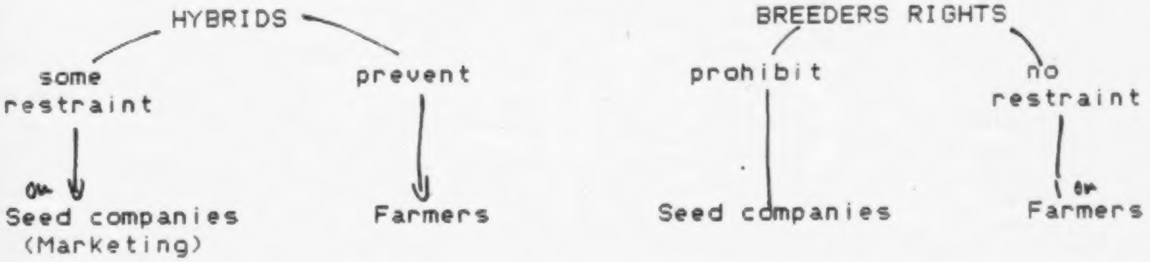
1. Hybrids, the only efficient method of protection

Hybrids prevent farmers from producing their own seeds without yield loss and create the necessary conditions for an oligopolistic market structure and indirect valuation of genetic resources and breeding work. They do not create an exclusive property right per se --at least with single crosses-- since inbred lines are widely distributed to farmers for seed production. Getting hold of the lines of one's competitors is easy.

On the other hand, breeders rights create direct property rights: they prohibit seed companies from producing seeds of varieties which are not theirs without agreement of the owner- but these rights cannot prevent farmers from producing their own seeds.

The following table shows the different rules of the game with different types of valuation of genetic resources:

COMPARISON OF METHODS OF VALUATION



The competition of farmers producing their own seeds has to be eliminated if any substancial profits are to be made out of a breeding work. Hence, direct property rights are not efficient from the standpoint of profits and this explains why more and more crops (including autogamous plants) are going the hybrid way even when breeders' rights are in force.

However, these two systems are not exclusive of each other. Soybeans somewhat combine the two systems: one the one hand, soybean seed is difficult to produce because the germ is fragile. In this regard, the soybean is similar to hybrid corn. On the other, soybean is an autogamous plant, and the genetic information can be legally protected. This unusual combination makes soybean the most profitable field of seed production after corn. When the Breeders' Protection Act was passed in the United states, soybeans naturally became the largest field of private research investment. In Europe, inbred lines and hybrids are legally protected whereas the United States, the hybrid seed corn industry was adamant against any system of direct hybrid and inbred line protection -very likely because it was so dependent upon public lines for its own hybrid production. It is now considering protecting some of its own proprietary inbred lines.

2. Systems of valuation and public research

A public breeder --when there are no breeder's rights in force-- will replace a variety only if he comes out with a better one. If his variety is outstanding and is widely used for years, so much the better.

A private breeder has first to make his company profitable. Doing so requires sales. And the strongest sale argument is to offer "new" varieties. Moreover, no seed company will advertise a non-proprietary item and when a new variety is offered, the only supply is from commercial sources. It is not surprising that statistics presented to Congress in 1980 to assess the effect of breeder's rights were "impressive": ...three to six times more varieties of wheat, soybeans, and cotton were produced in the decade after the passage of the 1970 Act than in the preceding decade. This is probably a case in which the patent system actually achieves the intended goal of increasing research investment." (Barton, 1982)

Varieties offered by private companies are very similar (US Congress, 1980). Since the 30's, it often happens in the US that identical corn varieties are offered for sale by different companies under companies' proprietary names. In Europe, public breeders who are on the committees that monitor the regulatory system fight with little success against the proliferation of similar varieties offered for sale.

Breeder's rights do increase oligopolistic non-price competition. What sort of agronomic progress flows from the new rule of the game and how efficient it is compared to the alternative of publicly funded breeding work cannot be answered with such figures.

More importantly, these figures overlook the structural transformation of breeding research entailed by the existence of a system of valuation. Public breeding is replaced by a division of tasks: public research does the non-proprietary work whereas private breeders use the work of public breeders and the genetic material coming out of their more basic research into their own breeding programs and proprietary commercial varieties. Public research gives up the production of commercial varieties as in Europe and the United States. The process of adjustment can be long, arduous, particularly for the older public breeders who may cling to their old ideal of serving the farmer whereas they have to serve now first the seed industry, hoping for the "invisible hand" to do what they used to do consciously. Budget restrictions may postpone the adjustement: this is the case in England where public wheat varieties still have 75% of the market in spite of almost twenty years of breeders rights. In the United States, hybrid corn research is an excellent illustration of this division of tasks and two outstanding public lines, Missouri 17 and B (Iowa) 73 are the basis a number of commercial hybrid crosses. In France, F2 and F7 two public lines and an american line very likely to be derived from a U.S. experiment station line enter the most successful present hybrid, Dea -not surprisingly a Pioneer hybrid. The official policy of INRA (Institut National de la Recherche Agronomique) is not to compete with private cereal breeders.

Over the long run, by gaining control over the rhythm of genetic progress, private companies will find it easier to develop hybrids in other species: when a wheat cytoplasmic male sterility was discovered in the mid 60's, companies scrambled to develop hybrid wheat -without success. While companies were struggling to back cross the CMS and restorer genes into promising lines for hybrid production -a process that takes several years-, public breeders were releasing better varieties which canceled out any advantage of hybrids. With breeders rights and a well entrenched division of tasks between public and private research, the simultaneous redirection of private and public breeding effort to hybrids would have made hybrid wheat easier. (*) If anything, the history of hybrid corn shows that the redirection of private and of public breeding to hybrids in 1921 -and hence the elimination of competition of open-pollinated varieties- explains their final success fifteen years later (Berlan, Lewontin, 1985).

3. The future of an unprincipled system

Breeders' rights are often described as a matter of principle: anybody has a "natural" right to get a reward from his efforts. However, the case of plant protection in the United States shows that such a right is historical and relative.

The first protection system was a patent law in 1930 for vegetatively propagated plants. Yet, potatoes were excluded from the protection under the presure of potatoe producers. The plant protection act of 1970 excluded 6 species from protection: celery, pepper, tomato, cucumber, carrot, okra. The frozen food and canned soups manufacturers feared it would increase their seed costs.

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* Other reasons contributed the failure to develop hybrids
cf. Berlan, 83, annex 2 and note 3

This denial of protection drove breeders of vegetable seeds into breeding hybrids -which they would have done anyway. Frozen foods and canned soup manufacturers saw their seed costs soar (hybrid seeds cost 10 to 15 times more than non hybrid seeds) while without breeders rights, they could supply non-hybrid seeds to their farmers under contract without paying any royalty to the breeder of the variety. Hence, in 1980, breeders' rights suddenly made sense to get less expensive seeds or to protect some eventual proprietary material coming from the companies' research investment. The new seed Act now includes these species.

The slighter the difference used to distinguish two varieties, the less efficient is the protection (Schmid, 1983). Lastly, in Europe, it was proposed that well-known genes would not any longer be used to distinguish a corn inbred line from another. For instance, a gene of silk coloration could not be back crossed into a successful proprietary inbred line to create a legally new line. Such a step would introduce the notion of gene into the system of protection. Why then not patent the gene of earliness discovered a few years ago in corn? Or genes of desease resistance? It shows that there is a strong temptation for a system of protection to encompass more and more, even if it is at odds with the objective of the drafters of UPOV Convention to severely restrict what could be protected.

At the 1980 meeting of F.I.S (International Seed Federation) the President of the cereal section refused to allow multiple maintainers of a single variety: if a variety was, say, maintained in the north of France, it would develop some frost resistance, while the variety maintained in the south would develop some disease resistance. Hence the variety would ultimately evolve! According to this view, the only perfectly stable and patentable form of life is a dead one!

CONCLUSIONS: MAKING A PUBLIC GOOD PRIVATE?

Except for hybrids varieties, production implies a diffusion of genetic information which is available to anybody and becomes a public good . The case of micro-organisms is different since the commodity sold is not the living thing but the product of the living thing once it has been killed. Between the fermenter and the pill or injection, more or less numerous and complicated steps occur (marketing not being the less important!) and the customer does not compete directly with his supplier. Still, the genetic information circulates easily and the folklore of the pharmaceutical industry is full of stories of visitors carefully wiping their noses and sending back the tissues with the precious strains of micro-organisms to their own labs. The point is that the good we are dealing with, genetic information , is difficult to control for one's exclusive use and tends to circulate freely.

Those who have been promoting breeder's rights in Europe, in the United States or elsewhere, as well as those who are trying to patent other forms of life have not paid attention to the innate contradiction of what they do. Limiting the use of a good available in limitless quantities at no cost will limit the full use of biological potentials to only what is patentable, will erect barriers to entry in branches of production where competition would be necessary. It is also our contention that tempering with the most fundamental characteristic of life, self-reproductibility, either legally or biologically neither leads to social or economic efficiency and will in the long run prove a fetter on the full use of the potential of present biological breakthroughts. The intrinsic public good nature of genetic resources should be recognized and accordingly, institutional measures should be taken. Hence the social value of genetic resources would not be any longer confused and plagued by the question of their valuation as commodities even if they can be enbodied into commodities. The ground would be cleared for a serene debate on their collection, evaluation, and use. The idea of their "common property" would concretely begin to take substance.

The present situation is similar to the one at the beginning of the 20th century when the matter of breeder's rights began to be discussed. By using the existing legal and institutional system, it was possible to develop a de-facto system of protection without legislative intervention. At present, by using the existing legal system, the patenting system has been extended to micro-organisms without due intervention of the legislator, although it was never intended to encompass such developments.

One may wonder what would have happened if the "Petition of Candle Makers and Associated Industries Against the Unfair Competition of the Sun" with its request that all opening of houses and buidings be covered, had been taken seriously. Frederic Bastiat's witty pamphlet to deride the anti-free traders of his time applies also to genetic resources in the late 20th century.

ANNEX 1

An analytical framework of the economics of the seed industry (non hybrid seeds)

Let:

P_g be the price of grain (per bu).

P_s be the price of seed (per bu).

C_u be the cost of processing the grain into seed on a processing plant (per bu).

C_f be the cost (bu) of processing the grain into seed ont the farm (per bu). This, in fact is a perceived cost including both the opportunity of foregone interest and erroneous views about the superiority of commercially produced seed that led the farmer to overestimate his actual seed production cost.

S be the premium paid by seed companies to farmers under contract (per bu)

T be the costs of transportation of the grain form the field of the contracting farmer to the processing plant and of delivery (per bu). These costs are important for most grain and oil seed crops -wheat, barley, soybean..., but are negligible for maize.

M be the maximum potential margin, that is what is left once the necessary costs of producing the seed are covered.

R be the royalty paid by the seed company to the breeder (but not by the farmer who produces his own seed).

A farmer will buy commercial seed only if its price is smaller than the price of the grain he has harvested and which he will withold from the market plus the cost of processing the grain into seed on his farm.

(1) $Ps < Pg + Cf$

A seed company will sell its production at a price that covers its direct production costs (procurement, transportation, processing) and still leaves a margin M to pay overhead, advertising and selling expenses, taxes and to provide profits.

(2) $Ps = Pg + S + T + M + R$

By replacing and rearranging, we have:

(3) $M < (Cf - Cu) - T - S - R$

in which $(Cf - Cu)$ represent the economies of scale.

If E is the economy of scale in the processing of the grain into seed, (3) can be written:

(4) $M < E - T - S - R$

The margin/bu depends upon the difference between perceived processing costs on the farm and the cost of processing in a seed company which is a definition of the economies of scale. Whether or not the seed industry can increase the perceived cost by advertising, varietal obsolescence, institutional and legal obstacle to seed production and saled by farmer is a practical question which depends upon many factors. One such factor is the policy of public research and extension service which can: a) actively organize farmers and teach them how to produce good quality seed in order to keep production costs low. This means a conflict with the seed industry; b) be indifferent and let te farmer decide by himself the best course of action. Marketing will then increase the perceived cost of on-farm seed production but it remains to be seen by how much; c) promote commercial seed for various reasons.

Annex 2

The economics of hybrid seed production

Hybrid seed companies fulfill three functions: they produce the inbred lines, they organize hybrid seed production through a network of farmers under contract, they process the grain harvested into seed and distribute it. A fourth function has been added from the very beginnings: the creation of new varieties.

Let us call:

YF1, YF2, YF0, the yield of the F1 hybrid, of the F2 generation and of the F0 generation (that is the seed yield, or the yield of the maternal pure line). (lbs/acres)

Ps, Pg, Pp, the price of seed, the price of corn for grain, and the price paid to seed producers under contract with seed companies (per bu).

RF1, RF2, seeding rate (pounds of seeds sown per acre).

Cm, Cf, costs of seed processing (cleaning, sorting, bagging, storing etc in a factory and at the farm level (per bushel).

S premium paid to seed producers by seed companies (per acre).

Ws Weight per kernel.

D Plant density in kernels per acre.

A seed companies sells hybrid seed to farmers at a price which covers the cost of producing the seed through its network of farmers under contract, plus the processing costs, plus its margin. The margin is what is left to the companies once their necessary costs of production are covered. It will be used to cover sale expenses, advertising, overhead, taxes, research and development and will hopefully leave a profit.

$$(1)\ Ps = Pp + Cm + M$$

If a seed company wants farmers to produce hybrid seed corn for them, they must guarantee that the income they receive is at least equal to the income they would get from growing ordinary corn plus a premium to cover the special expenses of seed production. (Although seed companies prefer to organize detasseling themselves, for the sake of simplicity we will consider that it is done by seed producers and is included in the premium paid to farmers).

$$(2)\ Pp = Pg\ (YF1/YF0) + (S/YF0)$$

Farmers have two options:

a. If they buy seeds from seed companies, their income will be:

Pg YF1 - RF1 Ps - production costs of corn for grain.

b. If they choose to use their own seeds, their income will be:

Option (a) is chosen if it gives a higher income. This can be written:

(3) $Ps < Pg\ (YF1 - YF2)/(RF1) + Cf\ (RF2 + RF2)/(RF1 + RF1)\ Pg$.

We may assume that for the period under scrutiny where all hybrids were double crosses $RF1 = RF2$

Last, by simple substitution, we have:

(4) $M < Pg\ (YF1 - YF2)/(RF1) - (YF1)/(YF0) + (CF - Cm) + (S)/(YF0) - Pg$.

(4) gives the upper limit of the margin of the seed industry. The lower limit corresponds to the costs of producing the seed, that is $Pp + Cm$.

Thus the margin of the seed companies is set between a lower limit, the cost of producing the seed, and an upper limit set up by equation (4).

(5) $Pp + Cm < M < Pg\ ((YF1 - YF2)/(RF1) - (YF1)/(YF0) + (Cf - Cm) - (S)/(YF0) \quad - Pg$

The second member of equation (5) shows that the maximum potential margin depends upon:

- the price of corn,

- the yield increase due to commercial hybrid seed (as compared to alternative sources of seed corn),

- the seeding rate (it is obvious that this is a crucial factor and that if the same yield increase "due to hybrids" is brought about by smaller quantities of hybrid seed, the per bushel value of hybrid seed is increased),

- the ratio of yield to seed yield of the maternal line;

- Cf-Cm are the economies of scale in seed processing and the higher they are, the higher the margin;

- the last factor is the per bushel of seed premium given to the seed producer. The interest of the seed companies is to keep it low.

Equation (5) shows that the new technique opened up a vastly increased field for profits compared to the previous situation with open-pollinated varieties. With these varieties, the grain harvested is genetically a seed, it suffice to make $YF0 = YF1 = YF2$ to have the equation of the margin of the seed industry in case of non-hybrid seed.

(5 bis) $M < Cf - Cm - T - S$

We find the previous equation (Annex 1) without the royalty rate which could be added.

At present, if we consider that the economies of scale offset the per bushel premium (not an irrealist assumption) and if we take Pg = 3 dollars, YF1 - YF2 = 50 bu, RF1 = 13 pounds, YF1/YF2 being now very small and negligible, then the maximum potential margin appears to be ...640 dollars per bushel. With present corn seed yields of 60 to 80 bu/acre, the per margin acre of hybrid seed corn production is potentially worth between 36,000 and 48,000 dollars and this after the necessary cost of production have been paid! Of course, this is only potential. If a seed company has a monopoly over the supply of hybrid seed, this is theoretically what it could charge plus or minus a dozen dollars to take into account the factors that we have neglected. If we have perfect competition, then the price charged for hybrid seed will be much lower and will cover the costs of production plus a normal profit.

This equation explains the failure to produce hybrid wheat, barley or soybeans (note 3). Since RF1 = RF2, the first member of parenthesis can be written as Yf1/Rf1 - YF2/RF2. This is the difference in the multiplication rates between the hybrid and the F2 generation . The multiplication rate (computed nationally from Agricultural Statistics) in the case of wheat, barley or other cereals, is small compared to maize: around 25, instead of 500 times The potential unit margin in hybrid seed production of small grain will remain 20 times smaller than for corn. The potential physical market for seed may be large but the unit margin will be much lower than for maize --unless tillering inducing chemicals increase the multiplication rate.

NOTES

(1) This requirement is absurd since once the variety is created, full disclosure of the methods and steps of creation is unnecessary. It illustrates the specificity of genetic information as a factor of production.

(2) The U.S. laws leaves open the possibility of a compulsory certification at breeder's request. The main difference is the absence of a catalog and of prior testing of varieties. But the State Experiment Stations yield tests to a certain extent replace this procedure. Hence, the differences between the US "open" system and the European "bureaucratic" one are not as important as the US seed industry wanted Congress to believe. In the United States farmers can sell their grain as seed but cannot advertise it -which severely limits sales! The law is ambiguous about farmers processing collectively their grain into seed, or having it processed for a fee, or trading their seeds through a clearing house to avoid to grow, harvest and process small quantities of each cultivar. It is likely that a strong pressure will be exerted in the future to confine seed production within each individual farm so that market for commercial seeds expands.

(3) Even if the seed production problem can be solved (that is even if YF1/YF0 can be made low), even if wheat or barley exhibit a strong "hybrid vigor" (which is not the case), as long as the multiplication rate is not increased, the profitability of F1 hybrids will remain for other species much lower than for corn. All the attempts to "hybridize" small grain crops will meet this obstacle which incidentally might be overcome with the use of tillering inducing chemicals, a development well in line with the consolidation of the seed industry into the pharmaceutical and petro-chemical industry. One may note that all grain species for which hybrid seed production has been successful have a very high multiplication rate: this is the case of millet, sunflower and sorghum.

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Technologies to Maintain Microbial Diversity

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TECHNOLOGIES TO MAINTAIN MICROBIAL DIVERSITY

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for

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ABSTRACT

Microorganisms are important in food and agriculture, health and welfare, industry, and national defense. The biotechnological revolution has increased the potential uses for the diversity represented in microorganisms, since desired traits can be decoded genetically and reconstituted in other organisms.

Culture collections house strains of microorganisms as the basic units of microbial diversity. The diversity of microorganisms is so great that it is virtually impossible to isolate and characterize all of the microbes from even a single ecosystem.

Natural ecosystems are rich sources of microbial diversity, and such *in situ* collections should be preserved. *Ex situ* collections are, however, a convenient and cost-effective way to store microbial diversity without the need to continually return to natural ecosystems.

The U.S. has several major culture collections, but differs from other countries with strong traditions in microbiology in that the U.S. government has not, to date, recognized microbial germplasm as a national resource that should be institutionalized with public support.

Excellent technologies are available for preservation of those microorganisms which can be isolated, but many microbes defy all attempts to isolate and culture them under laboratory conditions. Given these deficiencies and the necessity for additional research on the fate of microbial diversity in disturbed ecosystems, it cannot be stated definitively that microbial diversity is in jeopardy from human activities to the same extent as is the case with plants and animals.

Examples exist of microorganisms that have been lost from specific (polluted) environments, but there was no net loss of diversity because the species are represented in other environments. We have found no scientific evidence of pervasive loss of microbial germplasm. All indications are that microbial diversity is on the increase, though this may merely reflect that new techniques now make it possible to describe diversity more and more finely than before.

Although microbial diversity *per se* is not at risk, access to valuable microbial diversity cannot be guaranteed for the future without greater commitment to microbial culture collections.

This paper recommends formalization of a National Microbial Resource Network, with appropriate institutional linkages between the American Type Culture Collection and the Northern Regional Research Laboratory of the USDA to achieve its core management, service, and research functions. The network would extend to designated specialized culture collections throughout the U.S. and also to selected international microbiological resource centers.

GENERAL INTRODUCTION

Throughout history, people have been dependent on biological diversity in the environment for food, health, shelter, and even for much of their culture. At no point in history have people been as potentially capable of causing such major impacts on the environment that sustains them as is the case today. A combination of human population increase and advances in technological capabilities has brought us to the point in exploitation of our environment at which biological diversity is truly in jeopardy.

Extinction of rare animal and plant species is ready evidence that significant loss of diversity is occurring on a worldwide basis. The protection of threatened animal species, in particular, has proven a popular public cause. Endangered animal species benefit from the attention of numerous institutions and the investment of apparently abundant financial resources from public donations. Much of the public concern is no doubt fueled by a sense of identity with, and compassion for, animals faced with extinction.

Microorganisms will not enjoy public concern to anything like the same extent. Support for the preservation of microbial diversity will be advocated most frequently by an enlightened scientific community convinced of the key role which microbes also play with respect to people's food, health, shelter, and culture. One purpose of this paper is to focus attention of a variety of audiences on issues relating to the preservation of biological diversity of microorganisms.

There are two aspects to a full understanding of the importance of microbial diversity. One aspect is an appreciation of the degree to which microbes benefit mankind. The other aspect is recognition of the extent to which microbial diversity is changing, and how that change is being managed. Our paper concentrates on microbial diversity per se, but our entire treatise is predicated on acceptance by the reader that microorganisms are important -- just as important as animals and higher plants in this context.

The Importance of Microorganisms

There are many publications dealing with the importance of microorganisms. We will give only a brief overview here and provide citations for those who wish to read further into the subject.

Microorganisms are important, not only for their benefits, but also because of their harmful effects (Dixon 1976). Significant benefits of microorganisms include their contribution to soil fertility and crop production, their action in production of fermented foods and beverages, their capability for converting waste substrates to useful products, and their use as sources of antibiotics and other valued chemicals. Notable detrimental effects of microorganisms are the destructive influence of plant pathogens, the decomposition of structural timber, and the direct attack on human health and welfare by disease organisms.

Microorganisms are now the center of renewed research interest because of advances in our ability to manipulate them to advantage through recombinant DNA technology. Indeed, in the field of genetic engineering, microorganisms are essential because the production of recombinant nucleic acids, whether destined to be used in prokaryotes or eukaryotes, is accomplished in bacteria or other single celled microorganisms.

Those interested in further reading on the role played by microorganisms will appreciate the selection of papers on the theme, "The Microbe: Friend and Foe" presented in a special edition of Impact of Science on Society (Unesco, 1982).

Definition of Microbial Diversity

This paper will address the issues relating to biological diversity of microorganisms, paralleling to a large extent the approach that is being taken in the companion studies of diversity of higher plant germplasm. There are significant differences, however, not least of which is the way in which a unit of biological diversity is defined in the case of microorganisms.

Biological diversity is defined qualitatively as the richness and variety of life forms on this planet. A working definition of biological diversity which has been adopted for the overall OTA study is: "the genotypic and phenotypic variation found in populations of a single species, in addition to the temporal, structural, and spatial variations in communities of species and ecosystems." And since a key aspect of this study is determination of whether microbial diversity is increasing or decreasing, a quantitative definition is also in order. A definition from Mitchell (1974) is adopted for our purposes: the number of species that live together in an ecosystem.

Most people share a common understanding of the word "plant" and the word "animal." The term "microorganism" is less well defined. For the purpose of this paper, we shall consider the term microorganism as including any of the following: bacteria, including eubacteria and actinomycetes; cyanobacteria (blue-green algae); fungi, including yeasts; viruses: bacteriophage, plant viruses and animal viruses; vectors: plasmids and rDNA vectors; and tissue cultures: plant, animal and hybridoma lines.

Although this definition may seem artificially broad, all of these materials contribute to microbial diversity and warrant consideration as we review and anticipate technologies for the preservation of microbes.

In the future, some of the new technologies to maintain plant and animal diversity will be technologies more typically associated with preservation of microbial germplasm. This is to be expected when the vehicles of diversity that will be stored, namely cell and tissue cultures, resemble microbes in their maintenance requirements much more than their parent plants and animals.

STATE OF KNOWLEDGE OF MICROBIAL DIVERSITY

The diversity of microorganisms on Earth is immense. Only a very small fraction of microbes have ever been isolated and cultured, let alone identified or classified. An inherent difficulty in making a statement about our knowledge of microbial diversity is defining what constitutes a basic unit of diversity. In the case of higher plants and animals, the species is the logical unit of diversity. Changes in diversity at the population or ecosystem level are reflected in an increase or decrease in the numbers of species. As technologies become more sophisticated, more and more differences between individual organisms can now be identified. DNA homology and nucleotide sequencing procedures, for example, enable us to describe diversity even within species and this results in the species concept being something less absolute than previously regarded (Brenner 1983).

In microorganisms this problem is even more acute. The species is not necessarily the accepted unit of diversity. Individually identifiable strains of microorganisms are more often regarded as the basic units of microbial diversity. A strain is a population of microbial cells all of which are descended from a single cell, i.e., a clone (Brock 1979). Strains are isolated from natural environments and then stored as distinct entities in culture collections. Even though two or more strains may be very similar, except for their geographical location of origin, they are always maintained as unique accessions. Studies to quantify increases or decreases in microbial diversity are not as straightforward for microorganisms as they are for higher plants and animals, and this is a reason why very few such studies of quantitative changes in microbial diversity have ever been undertaken.

Evidence of Increase/Decrease in Microbial Diversity

A growing need to document the environmental impacts of industrialization, rural development, and other human endeavors has resulted in some studies to record the effects of pollutants on populations of microorganisms in natural ecosystems.

One case in point is the effect of air pollutants on lichen populations. Lichens are symbiotic relationships between various fungi, algae, and/or cyanobacteria, which live in a wide array of environments. Lichens are now recognized to be powerful indicators of air pollutants (Dixon 1976), because of their sensitivity to airborne contamination. By studying the prevalence of lichens in urban and suburban communities, an index of air quality can be derived (Gilbert 1965). These biological indicators can be lost completely from particular environments when air pollution controls are not instituted. Certainly this is an instance in which diversity within this environment is reduced by the loss of lichen genera. Yet, the genetic diversity of lichens as a class of microorganism may not be reduced overall, since species and genera of the lichens in question are represented in other environments.

Another case in which changes in microbial diversity might have come to light is the effect of oil or organic man-made pollutants on marine and estuarine microbial populations.

Pollution of marine and estuarine environments by oil or other man-made pollutants is generally perceived as having a damaging impact on the flora and fauna of the affected aquatic systems. It would seem reasonable to assume a reduction of microorganisms would occur in a polluted environment. This is not necessarily borne out, however, by data from microbiological investigations of instances of such pollution. Indeed the opposite is true, pollution has been shown to increase the numbers of, and selection for, certain types of microorganisms (e.g., hydrocarbon utilizing) in the affected environment. The increase in microbial diversity comes about because oil and many other pollutants can be metabolized by some microorganisms. These unusual sources of nutrients for microorganisms have lead to many new strains being selected or discovered in polluted settings which would not be found in pristine environments. Oil and related pollutants have not been shown to inhibit the growth of the normal microbial flora present prior to the pollution event (Walker and Colwell 1975).

Although pollutants have not been shown to reduce the numbers of microorganisms in an environment, pollutants have been shown to dramatically alter the composition of microbial populations within environments. Studies of microbial populations in the Atlantic Ocean at a pharmaceutical waste dump site have indicated that the selection of certain marine microorganisms over others in the population does occur as a result of pollution (Peele et al. 1981). This population dominance by a single or few microbial species may be extreme in such disturbed environments. In one such study, Vibrio spp. comprised almost 100% of the culturable marine bacteria (Grimes et al. 1984). Other bacteria, once considered common in this environment were found in only low concentrations. Although these bacterial species were dislocated from an environment, they did not become extinct, in the same sense that higher organisms might have if the environment changed so dramatically.

Clearly then, microbial diversity is not in jeopardy from human activities to the same extent as is considered to be the case with plants and animals. Polluted environments may actually contain greater diversity, reflected in a larger total number of species, but be dominated by very large populations of a select few of those species.

Such changes in the balance of species within populations can significantly alter the food chain. Ripple effects will extend also to populations of higher organisms because of their dependence on microorganisms as their food source. Thus, concern about degradation or loss of microbial environments will appropriately be a part of research on loss of higher plant and animal ecosystems.

Survey of Expert Opinion

It can be speculated that microbial germplasm which has not yet been identified, isolated, or cultured, may exist in natural environments and be lost unknowingly when those environments are eliminated or changed. This viewpoint is more commonly expressed with respect to higher plants and animals than it is in relation with microbial diversity (Wolf 1985). It is this concept that fuels the major programs underway to catalog and collect endangered plant and animal species (see Tangley 1985) before the fragile ecosystems which sustain them are lost.

We do not have enough data about microbial diversity to be able to assert that there is any microorganism uniquely associated with a specific environment to the extent that loss of that environment would lead to extinction. This situation reflects a need for additional research on microbial ecology, and is not yet a basis for stating that human impacts on the environment pose no significant threat to microbial diversity.

Recognizing that the way in which microbial diversity is defined virtually precludes performance of experiments to detect quantitative change in diversity, we attempted to assemble the opinions of appropriate individuals on this issue. The survey instrument was in the form of a letter (Appendix I). Responses which we received to this mailing (Appendix II) generally agreed with our premise that extinction of microorganisms _per se_ is probably not occurring. However, environmental change caused by mankind will induce fluctuations in the relative populations of microbial species. The general concensus among the scientists who we contacted was that only a small percentage of microorganisms have ever been recovered and cultured from a natural ecosystem. This is due to lack of knowledge about growth media, growth conditions and isolation parameters necessary to culture these organisms.

Since it is virtually impossible to collect absolutely all the microorganisms in a given environment with assurance, it may be far more important to conserve the environments themselves, rather than to try to assemble the inhabitants of that environment _ex situ_. This will be particularly true for symbiotic organisms like lichens which cannot be effectively conserved in laboratories. Collection, identification, and preservation of microorganisms should not be the only strategy followed to maintain microbial diversity. Natural environments bring about new microbial diversity by the mere fact that they are dynamic and are subject to continual change. Since microorganisms continuously adapt to new environmental conditions, diversity will increase over time.

New techniques in genetic engineering now make it possible for scientists to cause, or create, new microbial diversity (Dubos 1976). Most genetic engineering approaches involve identifying genetic codes for desired traits in one organism and copying the sequence for inclusion in other organisms. Outright creation of new gene sequences is technically feasible but lacks direction and will not be fruitful until molecular genetics is more thoroughly understood than at present.

Spontaneous mutation and the transfer of genetic material contribute to microbial diversity in natural environments (Reanney 1977). Manipulation of environments could, in theory, bring about artificially directed genetic drift. Again, our understanding of the factors at play is insufficient to permit use of such a strategy for accomplishing desired changes in microbial diversity effectively.

Microbial Eradication Programs

As important as collections of microorganisms will be in the future, the eradication of certain microorganisms from the earth is also being pursued vigorously. This microbial genocide (Dixon 1976) is being conducted in the interest of human health. Smallpox was last reported in a case in Somalia in October, 1977 (Behbehani 1983). In 1979, the World Health Organization (WHO) declared that smallpox had been eradicated from the world after an intensive ten year program. Cultures of the causative agent, the poxvirus *Variola*, are now held only by a few culture collections collaborating with the WHO. The question remaining is whether to continue to maintain the virus, or to attempt to destroy it totally (Foege 1979). Additional epidemic disease organisms will be the subject of eradication programs in the future. It is not clear at what point such eradication programs should stop, or whether all "undesirable" microorganisms ought to be annihilated.

Decisions about keeping cultures of pathogenic microorganisms for posterity will probably rest with national governments, since such microbes are considered incipient biological warfare materials. Detailed treatment of issues surrounding the potential use of microorganisms as agents of biological (germ) warfare is outside the scope of this paper. Such potential uses do illustrate, however, how special traits of some microbes might be sought after, and if found would raise the issue of policy and regulation of this special extreme of microbial diversity. In the U.S., there is no single governmental body which addresses the preservation of microbial germplasm, whether that germplasm is considered beneficial or harmful (Carlson 1976).

Even in the relatively less controversial case of agriculture, there is ample evidence already that management and disposal of harmful microbial genetic diversity is not a simple matter.

Agrobacterium is a plant pathogen, the causative agent of Crown Gall disease. Though this might make Agrobacterium a candidate for annihilation, to have done so would have deprived today's genetic engineers of one of their most powerful tools, the Ti plasmid.

Releasing new diversity into the environment, even when the purpose is to protect crops from frost damage, has resulted in one of the most embroiled regulatory entanglements in the history of microbiology.

These examples illustrate that the disposition of harmful microbial diversity on the one hand, and the promulgation of new beneficial microbial diversity on the other hand, are complex issues that extend beyond the domain of scientists and call for the formulation of policies that protect the public interest.

Specific Needs Requiring ex situ Storage

We have been unable to locate sufficient documented cases of loss of genetic diversity within given ecosystems to be able to describe loss of microbial diversity as "pervasive." Thus, pervasive loss of microbial diversity is not a defendable rationale for ex situ storage. The primary rationale for the assembly and proper upkeep of ex situ collections is that this is less costly than repeatedly returning to in situ sources of germplasm. A second and equally important rationale is the necessity of a reference collection for taxonomic and comparative microbial research. All biological research is based on comparative studies between different organisms, i.e. studies demonstrating the variety of morphologies, physiologies and potentialities of organisms. In microbiology, a "type" strain of a microorganism is designated which can always be used as a reference and through which all subsequent studies can be derived (Gibbons 1984). It would be impossible to reisolate type strains from natural environments each time comparative studies were initiated (see Holmes and Owen 1979 for an interesting case in which type cultures had been lost and attempts were made to reisolate the original strain again).

Current collections, many of them threatened for lack of financial support, represent an enormous prior investment of the time of experts and also of money. These collections are being actively used as resources to the scientific research community and to industry. In the absence of such collections, there would be a recurring need to return to the natural environment to re-isolate materials from the wild. And while in situ collections in their natural state remain an invaluable and perhaps preferred resource, the most cost effective approach to providing ready access to diverse ranges of microorganisms is to preserve the quality and assure the continuation of ex situ collections. If collections are neglected and lost, to begin again to collect the materials in today's economy would cost many times the original outlay, and there is no guarantee that specialists with the required expertise would even be available.

For the purposes of this report let us consider a hypothetical case study comparing the cost-effectiveness of an ex situ collection versus an in situ collection. The Hypothetical Culture Collection (HYCC) is a large in situ collection of 50,000 accessions. Let us assume that it distributes one fifth of its strains in any one year (i.e., 10,000 strains). Let us further assume that an average of US$ 75 is required to culture, maintain and authenticate each strain per year (this is a very conservative figure) amounting to a total cost of US$ 3.75M for the collection each year. To break even (and assuming no financial support from private or governmental sources), HYCC must charge US$ 375 for each of the 10,000 strains it distributes each year.

In contrast, Micro-Find, our hypothetical company which also distributes 10,000 microbial strains per year, does so by collecting desired strains from in situ environments upon demand and maintains no cultures in a collection. To estimate the cost of finding and isolating strains we have chosen to re-isolate two well-known and scientifically important Rhizobium strains from their locations of origin, one exotic and one domestic. We expect that by averaging the costs of isolating these two strains we can arrive at a rough estimate of the per strain cost of providing strains on demand.

The first strain, CB 756, is a broad spectrum strain, widely used for inoculating tropical legumes, that was isolated from Zimbabwe (Halliday and Somasegaran 1984). We have estimated that to travel to Zimbabwe, collect material, return to the laboratory (Dayton, Ohio), and isolate this strain would roughly cost US$ 4422. This is a conservative figure, since it presupposes that we will have no difficulty locating suitable material to collect and also have no difficulty actually isolating the strain.

The second strain, USDA 110, is a particularly competitive Rhizobium strain, used widely for inoculation of soybean, and originally isolated from Florida (Halliday and Somasegaran 1984). Again we have estimated the cost of travel, collection and isolation of this strain (assuming no difficulties), and obtained a cost of US$ 860. By averaging the costs of these two strains, we have derived a mean cost of US$ 2641 to isolate any microbial strain.

Returning to our hypothetical company, Micro-Find must charge US$ 2641 for each strain it distributes, and therefore, the necessary annual operating budget, assuming no profit on the 10,000 strains nor any financial support from private or governmental sources, would be US$ 26.4M.

From this simple example it is clear that in situ collections are far more cost-effective than constantly returning to the natural environment. Factors such as loss of natural environments, inaccessibility of environments due to climatic or political restraints, or difficulties in reproducibly isolating microorganisms further justify the role of ex situ culture collections. This is only true, however, if such collections are soundly managed, accessible to users, and have assured continuity.

MICROBIAL CULTURE COLLECTIONS

In 1972, under the sponsorship of Unesco, the World Health Organization (WHO) and the Commonwealth Scientific and Industrial Research Organization (CSIRO) of Australia published a directory of institutions in the world maintaining culture collections. In 1982, this directory was revised and updated (McGowan and Skerman 1982) and remains the primary, comprehensive source of information concerning international microbial culture collections. In addition, the American Phytopathological Society convened a select panel of scientists to discuss the importance and future of microbial culture collections (American Phytopathological Society 1981). Information from these sources indicates that there are probably 1,200-1,500 collections throughout the world. A brief history of several of the more important world collections is provided by Porter (1976).

Large Culture Collections

The largest culture collections (i.e., those with 10,000 accessions or more) are summarized in Table 1. It is significant to note that of these nine collections, four are held in the United States. Other countries with long traditions of microbiological research (Belgium, Britain, France, Japan, and The Netherlands) have one major collection each. Collections in this category may hold many different classes of microorganisms (LILLY, NRRL, ATCC, IP, IFO), or may be specialized for one or more taxonomic or functional groups (fungi: CBS, MUCL, IMI; medical: NRL). Of the large U.S. collections, only one maintains a catalog (ATCC), and could, therefore, be considered a resource collection of national importance (American Phytopathological Society 1981). The USDA/SEA Culture Collection (NRRL), although the largest public collection in the U.S., is an archival collection where cultures are stored away for long-term preservation. In actual distributions of cultures, ATCC far exceeds NRRL each year, although ATCC is a smaller collection and charges a fee. This is due in part to the fact that NRRL, as well as the other large collections, does not supply a catalog, and does not encourage general distribution of strains.

Some culture collections make it their policy not to distribute strains (e.g., LILLY). One reason for such a policy is the high cost of distributing strains and maintaining current records in catalog form. Another reason stems from a need to protect elite proprietary strains, some of which are patented. The American Type Culture Collection, the only large, generalized collection to distribute strains on a grand scale, can manage to do so by charging a distribution fee (currently $40/culture non-profit; $64 industrial or foreign). This fee recovers the cost of distribution as well as documentation (Gherna et al., 1985).

Moderately-sized Collections

The next arbitrary grouping of culture collections which we have summarized are those with holdings of between 3,000 and 10,000 accessions (Table 2). Most of these collections are sponsored by their respective governments, and unlike U.S. public collections, maintain catalogs and distribute strains on a fee or exchange basis. These collections are similar operationally to the American Type Culture Collection. The only U.S. collection represented in this group is that of the Upjohn Company (UPJOHN) which distributes strains only under limited circumstances. No publicly supported culture collection in the U.S. plays the role that national collections of this size do in other countries. It is perhaps the very existence of the ATCC that has prevented the establishment of a publicly supported resource collection in the U.S.

In Table 3, we have summarized the functions of all the large and moderately-sized U.S. culture collections. It should be noted that the primary archival and reference collections in the U.S. are the NRRL and the publicly-supported culture collections.

The ATCC, a living resource collection, and the NRRL, are the largest collections of their type in the world. As in many other aspects of the Life Sciences, the U.S. plays a major international role in the collection and preservation of microbial germplasm.

Specialized Microbial Collections

The remainder of collections listed in the World Directory (McGowan and Skerman 1982) have holdings well under 3,000 accessions and are usually limited to a specific taxonomic group or special purpose. Small, specialized collections of this type are often personal collections of individual scientists and may receive little or no direct financial support. Examples of these small specialized collections are discussed further in this section.

Specialized microbial collections usually begin as personal collections of microorganisms that a scientist working in the field accumulates and maintains over his or her career. One example of a specialized collection is that of the *Frankia* culture collection held at the Battelle-Kettering Laboratory in Yellow Springs, Ohio. This collection began as a personal commitment by Dr. Dwight Baker to the isolation and culture of frankiae, the symbiotic actinomycetes of nitrogen-fixing, actinorhizal plants. Dr. Baker's collection is highly regarded internationally, but is typical of the many personal collections that are not backed by any institutional commitment or extramural support. This makes the collection completely dependent on the dedication and resourcefulness of its curator. Such collections are vulnerable to deterioration if funding cannot be identified for their upkeep, and they may eventually be lost (Dowler and Fisher 1983).

The ATCC, though now much changed and streamlined, also began as a modest collection started by C.E.A. Winslow at the Museum of Natural History in New York (Clark and Geary 1973).

Specialized collections usually hold only a limited number of microbial genera but, because they are unequalled for depth within those genera, they are an important facet of the total microbial diversity conservation effort. The disadvantage with these collections is that upon the retirement or death of the scientist who curates, the collection deteriorates and is lost.

The case of the O.N. Allen collection of Rhizobium provides an interesting illustration of the factors at play in determining the fate of vulnerable personal microbial collections.

The Allen collection was assembled by the husband and wife team of O.N. Allen and Ethel K. Allen over the course of their careers with the University of Wisconsin. Professor Allen's worldwide collection expeditions, including significant coverage of tropical ecosystems, and his characterization of many of the isolated strains of Rhizobium while at the University of Hawaii resulted in the richest assembly of germplasm of this class of soil bacteria in the world at that time.

The collection remained directly dependent on Professor Allen's interest, and when he died in 1975, the University of Wisconsin was unable to provide for future management of the collection. This was not an unreasonable position for the University of Wisconsin to take, given that maintenance of the relatively large collection would have required a substantial time commitment by technical and professional staff to maintain it to a high standard. At that time, the collection had not been fully characterized or cataloged, and this further reduced the incentive for the University of Wisconsin to accept the burden of its upkeep.

In the wake of the energy crises of 1973/74, the University of Hawaii was contracted by the United States Agency for International Development (USAID) to conduct research, training and international outreach on renewable energy fertilizer technologies based on the legume/Rhizobium symbiosis. As part of this effort, a germplasm resource of the tropical rhizobia was to be assembled. The University of Hawaii was approached to accept responsibility for the Allen collection in 1976 and did so, contingent on continued extramural funding of the NifTAL Project.

A home had, therefore, been found for the collection -- but not a permanent one, given the temporal nature of USAID funding. In 1981, the University of Hawaii was asked to accept designation as a Microbiological Resource Center (MIRCEN), under the auspices of Unesco and UNEP/Unesco/ICRO Panel on Microbiology. Designation as a MIRCEN was conditional on the University of Hawaii committing to preserve the Rhizobium Resource in perpetuity. This commitment was not undertaken lightly, and in signing the agreement with Unesco, the University of Hawaii set about transfer of the entire collection to lyophilized form within the life of the USAID funding to the NifTAL Project, so that the maintenance costs would be minimized later when the University would become solely responsible for its upkeep.

In 1984, the catalog of the Rhizobium Germplasm Resource at NifTAL was published (Halliday and Somasegaran 1984). Over 1,700 strains were documented, including a substantial block representing the original Allen collection. Thus, the investment of Professor Allen's time and talents to assemble in the rhizobia into his original collection continues to benefit, and be fully accessible to, future generations.

Vulnerability of Collections

Like many scientific institutions currently, the culture collections are suffering from financial problems. Their plight is even more acute, however, because their function is not readily apparent. They do not have the glossy attraction of other scientific fields like biotechnology or genetic engineering. Funding for the U.S. Department of Agriculture is being reduced or redirected to "growth" topics and, as a result, the network of archival culture collections (e.g., NRRL) is receiving imposed funding reductions. Commercial culture collections, i.e., ATCC, must maintain their collections strictly on a no-loss basis. Accessions which are not used frequently and have no current intrinsic value are subject to being discarded or lost through neglect.

An even more severe problem arises when lack of funds for culture collections diminishes their ultimate usefulness because of reduction in staff time for record-keeping, authentication, and taxonomic characterization (Dowler and Fisher 1983).

Occasionally, but without regularity, very important personal collections are acquired and accessioned by larger culture collections. NRRL has acquired the Naval quartermaster collection and the collection of Selman Waksman (Nobel laureate for the discovery of streptomycin) in this manner. ATCC has acquired collections in this way also (e.g., the Naval Biological Laboratory Cell Bank Collection). Funds are, however, restricted at these large institutions and there is a limit to the number of small collections which they can assume.

In 1975, three international organizations, the United Nations Environmental Programme (UNEP), the International Cell Research Organization (ICRO) and the United Nations Educational, Scientific, and Cultural Organization (Unesco), recognized the need for moderately-sized culture collections, each which had a specialized purpose and belonging to a network of collections around the world (Colwell 1983). The establishment of microbiological resource centers (MIRCEN's) began at that time and such specialized resource collections are now located in nine locations, Brisbane, Australia; Stockholm, Sweden; Bangkok, Thailand; Nairobi, Kenya; Porto Alegre, Brazil; Guatemala City, Guatemala; Cairo, Egypt; Paia, Hawaii, USA; and Dakar, Senegal.

Collection Gaps and Collection Priorities

As mentioned above, we have not been able to find evidence of pervasive loss of any microbial diversity. Once again this reflects our lack of a comprehensive knowledge about the total microbial diversity of any one environment. We will not be able to acquire such a knowledge of even one or two currently "endangered" environments in a readily definable time period because of our inability to isolate, culture and characterize a majority of the microorganisms in a specific environment. Even with total microbial characterization of endangered environments as a research goal, humanity will never understand all ecosystems before they are destroyed, because of the inherent complexity of microbial populations and interactions. Therefore, to suggest that a few specific environments should be preferentially given priority and characterized for microbial diversity as a prelude to ecosystem exploitation is unreasonable.

Collection gaps probably do exist, but more from our lack of knowledge than from a lack of effort. Therefore, it will be more appropriate for research efforts to be directed to several generalized research topics which will improve capabilities at all collections and increase our knowledge of microbial ecology. In addition, microbial collections should be encouraged and financially supported to accession more and more varied microorganisms in their holdings and, in particular, microorganisms generally considered difficult or fastidious. In this regard, the first research topic deserving attention is research on isolation methods. Microorganisms which are not readily isolated and cultured are enigmatic because science has not identified some environmental requirement and duplicated that in the laboratory. Some microorganisms, even when isolated, grow only marginally in the laboratory. Research into microbial culture could perhaps allow us to characterize microbial diversity in natural environments more thoroughly as well as more efficiently handle difficult microorganisms in existing collections.

Another research priority is that of microbial synergism which permits efficient functioning of the microbial flora of an environment and ultimately supports higher organisms in that environment. Microorganisms do not live in sterile environments devoid of other microorganisms. Interactions and dynamics between microbial populations influence the entire biological food chain of an environment. Thus, research into microbial ecology at this level is an integral part of any strategy to preserve higher organisms.

A further priority is that of finding microbial indicators of environmental destruction. Endangered species of all types are subject to extinction because the environments in which they live are destroyed. Loss of plant or animal species can be readily determined even by the lay scientist. But such loss is undoubtedly preceded by some more subtle change in the environment, e.g., pollution or nutrient loss, that could be detected far earlier in the microbial population because microorganisms are the first to be influenced in the food chain. Fluctuations in microbial composition in ecosystems could forewarn of impending environmental destruction that would be critical to higher organisms. Just as lichens are currently being used to determine the amount of air pollution in the environments in which they exist, other microorganisms could become biological indicators for environmental protection and management.

TECHNOLOGIES TO PRESERVE MICROORGANISMS

The purpose behind the preservation of microorganisms is to maintain a strain for an indefinite amount of time in a viable state equivalent to that when it was isolated from its environment. The continuous culture of a microorganism is one way to accomplish this but it is expensive both in materials and labor and does not ensure that the genetic stability of the microorganism will be maintained. Therefore, techniques for long-term storage have been established which can approach the theoretical aim of preservation. Any preservation method attempts to reduce the metabolic rate of the microorganisms and, therefore, the rate at which the microorganism propagates and uses nutrients. All methods which accomplish this will cause the death of a certain percentage of the population of microbial cells. What is necessary, therefore, is that the method not reduce the viability of the strain to the point that no cells survive. An additional task of preservation is to maintain accurate records about the collection of strains and to document the history of the strain over time. This involves recovering preserved cultures over time, determining their viability and their purity: a procedure called authentication. This is a tedious and time-consuming task but is extremely important to the usefulness of the collection.

The majority of microorganisms currently being preserved in culture collections are held by lyophilizing (freeze-drying) or by ultra-freezing (Gherna 1981). These two methods permit the storage of microorganisms for extremely long periods of time (presently known to be longer than 30 years, Gherna 1981). Other methods for short-term storage of microorganisms which are used under some circumstances are immersion in mineral oil, low-temperature freezing, and desiccation (drying). A brief summary of each of the preservation methods is given below, with its advantages, disadvantages and cost-effectiveness. The information provided here was adapted from Gherna (1981).

Lyophilization

Lyophilization or freeze-drying is presently the most commonly used preservation technique within culture collections. Healthy microbial cells grown under optimal growth conditions are dispensed into small sterile vials or ampules at a relatively high concentration level (e.g., 10^6- 10^7 cells per ml). The vials are then quickly frozen in a super-cooled liquid solvent bath or in a mechanical ultra-freezer (-60°C). The vials containing the frozen suspension of microbial cells are then attached to a vacuum pump, either individually using a vacuum manifold or collectively using a vacuum chamber. The vials are held under high vacuum (20-30μm) until the water in the samples has sublimed. The glass vials are then heat sealed under vacuum using an air/gas torch to melt the glass at the top of the vial. Sealed vials containing the lyophilized cells are stored below 5°C. Lyophilized cells stored at lower temperatures (-3° to -70°C) in a mechanical ultra-freezer may have an even longer viability life. Cryoprotectants may be used to protect microbial cells from undue damage to cellular constituents during the freezing process. ATCC routinely uses 10% skim milk or 12% sucrose for cultures. Curators at NRRL, however, feel that serum (bovine or equine) is a better cryoprotectant for all microbial species. Each of these solutions will reduce the damage to the cell caused by ice crystal formation. Recovery of the lyophilized cells is simple and straightforward. The sealed vial is opened by scoring and then a small amount of liquid medium is added to the vial to rehydrate the cell pellet. The rehydrated contents are then transferred to a culture vessel containing an appropriate growth medium. Although the initial cost of equipping a laboratory to undertake lyophilization is moderately high (about US$ 5,000-10,000), the cost of actually preparing lyophilized cultures is very inexpensive. The long-term viability of lyophilized cultures is excellent and, therefore, this procedure is probably the most cost-effective means of microbial preservation in use today. Unfortunately, some microbial species do not fare well under lyophilization so other techniques for cell preservation must be used.

Ultrafreezing

Fastidious microbial species which do not retain viability under other preservation methods (e.g., plant pathogenic fungi) often can be preserved by ultra-freezing (Anon. 1983). In this procedure, cells sealed in vials or ampules are frozen at a slow cooling rate (1°C/minute) until they reach -150°C. The vials are then stored at -150° to -196°C using liquid nitrogen freezers. Cryoprotective agents, as for lyophilization, are necessary for this procedure but usually differ from the protectants described above. ATCC routinely uses a mixture of glycerol (10%), DMSO (5%) and culture medium for most bacterial strains. These chemicals are actually taken into the cells and protect it internally rather than externally like those chemicals used for lyophilization. Recovery of ultra-frozen cells must also be handled carefully. Viability of microbial cells has been determined to be best when thawing of the sample is done rapidly. The sealed vials are placed into a water bath at 37°C until all ice melts. The vials are then opened and transfers made directly from the sample. Ultra-frozen cultures must be maintained at the very low temperatures at all times during their storage and, therefore, the use of liquid nitrogen freezers with elaborate temperature controllers and alarm systems is necessary. Vigilance in being sure that each of the storage freezers is operating properly over long periods of time makes this procedure an extremely costly one. This technique is reserved, therefore, for microbial species which are not amenable to less costly procedures.

Immersion in Mineral Oil

Microbial cell cultures can be stored for short periods of time if the culture is overlaid with sterile mineral oil. The mineral oil prevents dehydration and reduces the metabolic rate of the organisms (Gherna 1981). Cells are grown on either agar slants, stabs or in broth cultures. After incubation and growth, mineral oil is added to the culture to a depth of about 2 cm. The cultures are then stored at about 4°C. Recovery of cells from these cultures is by inoculating loop or needle similar to normal subculturing. Although cultures preserved in this way have remained viable for up to 2-3 years, the method is not recommended for long-term storage.

Immersion in mineral oil is a very inexpensive procedure for short-term storage. However, if cultures have to be recovered, authenticated, and restored every few years, then it becomes costly in labor costs for long-term storage.

Low-temperature Freezing

Some microbial cells such as some of the actinomycetes and some soil-borne spore formers can withstand normal freezing processes and retain both viability and genetic stability. Cultures are stored on growth media at 0° to -20°C. Cells may remain viable for up to two years but damage to cells caused by ice crystal formation is thought to be extensive. For certain resistant microbial cells, low-temperature freezing is inexpensive and useful for short-term storage. Like immersion in mineral oil, repetitive recovery, authentication and restorage makes this technique labor intensive for long-term storage.

Desiccation

The majority of microbial cells die if they are desiccated (Gherna 1981). A few microorganisms, however, can withstand dehydration and remain viable for moderate amounts of time. Spore-forming bacteria are particularly suited to this method, Microbial cells are usually transferred to a sterile solid material and then dehydrated under vacuum. Soil, paper, and ceramic beads are frequently used. Cells may also be suspended in gelatin solution and then drops of the gelatin dried *in vacuuo*. Desiccated cells must be stored in desiccators and will remain viable longer if refrigerated. Recovery of the cells is by rehydration and subculture. This method is relatively inexpensive and is routinely used for some bacterial genera (e.g., *Rhizobium*). However, other techniques if available are preferred for long-term storage.

In an effort to compare the cost-effectiveness of long-term *versus* short-term storage of microorganisms, we have estimated the costs associated with storing strains by lyophilization and desiccation over a 50 year period. These two techniques are widely used for the storage of agriculturally important rhizobia at present.

For lyophilization we have hypothesized that the cultures will require subculture and authentication every 10 years. This is a rather conservative view since bacterial strains of this type are known to survive for at least 30 years, but for the purposes of this example we shall use the 10 year cycle. Desiccation on the other hand requires subculture and authentication at least every 5 years and probably more frequently, but for these purposes we shall use a 5 year cycle.

For each subculture and authentication cycle we estimate that 2 man-days of labor and a small amount of materials will be required, regardless of whether the strains are stored by lyophilization or desiccation. We suggest that at 1985 figures, approximately US$ 250 will be required to subculture and authenticate the cultures initially. Thereafter, we have included a 2% per annum inflation factor (again conservative) over the 50 year duration of our hypothetical test. Using the above cost estimates and assumptions, we have derived a cost of US$ 2,604 per strain for storage of lyophilized cultures and US$ 4,736 for storage of desiccated cultures for 50 years. Extrapolating to a hypothetical culture collection of 50,000 strains, storage by lyophilization over 50 years would cost US$ 130.2M, whereas storage by desiccation over the same period would cost US$ 236.8M.

This simple example demonstrates that any effort to increase the storage life of cultures will dramatically reduce the carrying cost of a collection.

Public vs Private Roles

Currently, the technologies necessary to conserve microbial diversity are in the public domain. It can be speculated that molecular biological techniques capable of creating diversity may become the proprietory technology of private companies (Demain 1976). Most authorities hold that this will not become a very serious problem because the research effort on these technologies in the public sector is on a par with that in the private sector, and the research discoveries will occur in both sectors. It can be expected, however, that unique genotypes with commercially valuable traits will be identified and retained as the proprietory materials of private sector companies. Indeed this is already the case and can be expected to increase.

TECHNICAL CONSTRAINTS

Tried and proven methods are available for the storage and use of most of the classes of microorganisms that it has proven possible to isolate into pure culture. These have been described in some detail in this paper. Methods for the preservation of DNA, RNA and perhaps even protein molecules that constitute genetic diversity, particularly with the increase of genetic engineering activities, may be necessary in the future. At present these molecules are incorporated in appropriate host cells (e.g., bacteria, plant or animal cells) and the host cells stored using the above techniques. This is an acceptable and reproducible method for long-term storage, however some efforts in the distant future may focus on storage of naked genetic molecules.

Therefore, within the United States and in most other industrialized nations, there are no major technical constraints limiting the storage and use of microorganisms. Developing countries lag industrialized countries in the application, utilization, and technological knowledge of microorganisms (Pramer 1984). Thus, the U.S. and other industrialized nations must shoulder responsibility for addressing the need to preserve microbial diversity.

Less developed countries are at a disadvantage when it comes to maintaining culture collections. The most satisfactory long-term storage techniques for microorganisms are also those which are technologically the most sophisticated. In the case of cryogenic cultures in liquid nitrogen, the technique requires a dependable power supply. Lyophilization of cultures in sealed, evacuated glass ampules results in culture units that have excellent longevity, even when stored at room temperature. The attraction of this method for developing countries is diminished slightly by the relatively high initial cost of equipment and problems keeping lyophilization equipment operational.

Improvization in the laboratories of developing country scientists has resulted in a wide array of variations of the preservation methods detailed in this paper. Though in many cases these methods are satisfactory to the individual collection curators, most require regular subculturing. This makes them suitable only for relatively small collections, and increases the likelihood of genetic drift.

The remaining challenge is to proceed with research leading to successful isolation of the majority of microorganisms that have never been cultured under laboratory conditions. Basic research into the isolation and cultivation of fastidious microorganisms is very much in the public interest and should continue. Deficiencies in this area have recently been highlighted by the outbreaks of human diseases which have attained almost epidemic proportions. In 1976, the outbreak of a highly fatal bacterial infection among attendees at an American Legion convention led to the discovery of an unusually fastidious bacterium, since named Legionella.

Even when the full human and technical resources of the Centers for Disease Control were applied to identify the source of the illness, it still took from July, 1976 until January, 1977 before the microorganism was isolated and characterized (Fraser and McDade 1979).

A similar health problem of even greater proportions and implications is the current epidemic spread of AIDS (Acquired Immune Deficiency Syndrome). The cause of this disease has not been fully determined, although it is likely to be a type of human retrovirus named the human T-cell leukemia-lymphoma virus (HTLV-III, Markham *et al*., 1984). Our knowledge of this virus is painfully inadequate, a fact which has lead to anxiety and hysteria in the general public (see Enlow 1984).

Legionellosis and AIDS will undoubtedly not be the last microbial diseases to achieve almost epidemic proportions in the world. Basic research into isolation, growth, and characterization of fastidious microorganisms, not only of clinical importance but of general importance, will enable the scientific community to better handle such outbreaks in the future. Such an approach will permit application of the proverbial ounce of prevention to microbial problems and reap a pound of cure in anticipating and handling such problems in the future.

Maintaining and distributing a current catalog is the goal of virtually every collection curator. Without a catalog to provide potential users with ready access to its contents, the value of a collection is greatly diminished. That very few collections are adequately cataloged is a reflection of just how onerous this task can be. In a sense this aspect of collection management has been constrained for lack of an appropriate technology. The advent of the microcomputer and highly adaptable, user-friendly database management systems software heralds a new era, enabling a curator to compile, output, and update a catalog inexpensively and with relative ease (Colwell 1976).

INSTITUTIONAL CONSTRAINTS

Mere existance of a satisfactory technique for storing microbial germplasm will not necessarily guarantee the systematic conservation of microbial diversity. None of the culture collections referred to in this paper (with the possible exception of the Rhizobium Germplasm Resource at NifTAL) was begun with the prime purpose of preservation of microbial diversity. The diversity currently represented in the world's major collections is a consequence of the research interests of the respective curators, rather than the raison d'etre of the collections. Appropriate institutional commitments and interrelationships will be necessary if a strategy for conservation of microbial diversity is to be workable.

A situation in which for-profit corporations hold large culture collections to support their business interests does not necessarily satisfy the scientific and societal functions expected of a national resource collection. Collections of this type, e.g., that of Eli Lilly, Upjohn, and others, are not fully accessible to independent users. Catalogs of the contents of these collections are not circulated and requests for cultures may not be serviced.

In the public sector, the NRRL collection would appear to be a logical entity to be responsible as a custodian of a national resource for microbial diversity. However, the staff at NRRL are research staff and are to devote the majority of their time to research studies on the collection. Therefore, they do not have adequate time or resources for preparation of an adequate catalog to give users access to the content of the collection.

The American Type Culture Collection is one entity in the United States that comes close to conformity with the role of a national resource collection. The sole business of the ATCC, a non-profit organization, is the collection, characterization, and distribution of elite strains of microorganisms (Gherna et al. 1985). Not all of the management criteria at ATCC will favor preservation of microbial diversity, however. It is conceivable that strains may be dropped from the collection when they cease to be in demand and become a financial burden in the long term. The preservation of microbial diversity is not assured under such an arrangement. Nor is there provision for active research programs on the specialized classes of microorganisms held by ATCC.

Given the record of successful accomplishment and high professional standards of the ATCC to date, consideration should be given to those mechanisms that might enable the ATCC to serve in a more formal way as a National Resource Collection, committed to the preservation in perpetuity of designated microbial diversity. This strategy, will be less costly than establishment of a public entity de novo to accomplish the same goal. Such a mechanism might be a Cooperative Agreement with a Federal Agency. By agreement, ATCC would be assured of stable continuing financial support, but be accountable to an independently appointed Germplasm Board for all actions impinging on the diversity of its holdings.

A considerably enhanced resource would result if the management and service strengths of the ATCC could be teamed to the research capabilities and broader germplasm sets at the NRRL. In a sense this is a proposal for a symbiosis of two very different, but complementary, organizations to better address the goal of establishing a National Microbial Resource Collection.

An extension of this concept would be to network the ATCC-NRRL team with designated, specialized collections to provide in-depth coverage of the holdings in which they specialize. Even though a network of this type would represent a significant management challenge, establishment of a National Microbial Resource Network should be explored.

Two main obstacles can be anticipated to establishing a National Microbial Resource Network. One is the differing histories, traditions, and the sense of independence of existing collections. The other obstacle is the difficulty of standardizing technical and informational protocols to assure meaningful interchange of germplasm and data among participating network institutions.

One network of microbiological resource centers, the MIRCENs, has been addressing these obstacles now for almost a decade. The World Data Center concept calls for a central clearing house for information on microbial germplasm held at MIRCEN collections and elsewhere. In practice, however, this endeavor has been frustrated by inability to come up with standardized data sets that reconcile the differing orientations of the curators of individual collections worldwide, and by financial resources that fall far short of the level required for the mammoth task of assembling the data base.

A more focused attempt to achieve an integrated microbial resource data base was initiated in 1984 by Unesco. The Inter-MIRCEN project is still in progress and provides for standardization of a minimum data set for characterized strains of Rhizobium, adoption of compatible data base management systems (based on the NifTAL model), and periodic publication of an integrated catalog of the collections held at Beltsville, Porto Alegre, Nairobi, and Hawaii.

If recommendations to establish a National Microbial Resource Network are acted on, the planning phase should include a close appraisal of the lessons learned in the international MIRCEN effort. While there may be reservations expressed about whether the institution building rationale for the MIRCEN program predestines the MIRCEN collections to be of less than optimal quality, the MIRCEN program has achieved, with limited financial resources, a high degree of network effectiveness, including regular global and regional newsletters, electronic data exchange, and computer conferencing.

ACTION PLAN

Our assessment of technologies to conserve microbial diversity suggests that an appropriate role for the U.S. Congress would be as follows:

- Affirm the importance of microorganisms in relation to human health and welfare, and recognize that even though most microbes are not visible to the naked eye, they are as diverse in form and function as are higher plants and animals.
- Assure that adequate attention to issues relating to microbial germplasm resources is included as an integral part of studies, research, and other programs dealing with biological diversity.
- Acknowledge that our understanding of the extent of microbial diversity in natural ecosystems is woefully deficient, and support research on microbial ecology (through grant programs and in-house research at NIH, DOE, EPA, and USDA).
- Appreciate the complexity of the technical barriers faced by microbiologists trying to isolate and culture fastidious microorganisms, and support basic research on microbial physiology (through grant programs and in-house research by NSF, NIH, and USDA).
- Accept that it is unrealistic to expect ever to be able to assemble the entire gambit of microbial diversity from even one of the world's threatened ecosystems and take steps to preserve in situ germplasm resources in reserves set aside for that purpose.

- Affirm the societal and scientific value of ex situ collections of microorganisms; recognize the significance of the role played by individual microbiologists, who historically have been the driving force behind assembly and maintenance (often with completely inadequate financial support) of the microbial collections of this country and around the world; recognize that these collections are becoming of ever-increasing utility in the biotechnological revolution; and provide direct long-term financial support to designated microbial collections.
- Enlist the support of major public and private institutions holding major microbial collections to proceed towards the formalization of a National Microbial Resource Network, the core of which would be comprised of the ATCC and the NRRL, the former fulfilling the role of management entity and handling service functions, and the latter dedicated primarily to research on the diversity of microbial holdings in the collections.
- Satisfy the needs of the less developed countries for access to microbial diversity by enabling (through USAID funding) the U.S. National Microbial Resource Network to circulate catalogs and technical newsletters to LDC counterparts, and to service requests for training, information, and strains of microorganisms.
- Recognize that the Microbiological Resource Centers (MIRCENs) play a key role as an international network appropriate for training and research on microorganisms of importance in international development, and advocate linkage with the MIRCEN network as a logical mechanism not only to extend the benefits of the U.S. National Microbial Resource Network to the rest of the world, but also to facilitate more effective assembly of diverse microorganisms from varied ecosystems worldwide.

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TABLES

Table 1. Microbial Culture Collections with Holdings Greater than 10,000 Accessions

Name and location	Type*	Accessions	Catalog	Notes	Acronym
Northern Regional Res. Laboratory, ARS/USDA, Peoria, IL USA	G	76,000**	No	free distribution to collections	NRRL
Eli Lilly and Co., Indianapolis, IN USA	I	66,000	No	no distribution	LILLY
American Type Culture Collection, Rockville, MD USA	NP	36,000**	Yes	distribution fee	ATCC
Institut Pasteur***, Paris, Cedex, France	NP	33,000	Yes	conditional distribution	IP
Neisseria Reference Laboratory, USPHS, Seattle, WA USA	G	30,000	No	free distribution	NRL
Centraalbureau voor Schimmelcultures, Baarn, The Netherlands	G	25,000	Yes	distribution fee	CBS
Mycotheque de L'Universite Catholique, Louvain-la-Neuve, Belgium	G	13,000	?	distribution on exchange basis	MUCL
Culture Collection of the Institute for Fermentation, Osaka, Japan	NP	11,000	Yes		IFO
Commonwealth Mycological Institute Kew, England, UK	G	10,000	Yes	distribution fee	IMI

* Type of collection: I = Industrial corporation; G = Government; NP = Non-profit or not-for-profit.
** Current number of accessions, obtained by personal communication
*** Institut Pasteur is divided into nine specialized collections which have been combined here.

Table 2. Microbial Culture Collections with Holdings Between 3,000 and 10,000 Accessions

Name and location	Type*	Accessions	Catalog	Notes	Acronym
The Upjohn Company, Kalamazoo, MI USA	I	7600	Yes	conditional distribution	UPJOHN
Plant Diseases Division Culture Collection, Auckland, NZ	G	7000	Yes	conditional distribution	PDDCC
Wellcome Bacterial Collection, Beckenham, Kent UK	NP	7000	Yes	conditional distribution	WRL
USSR Academy of Sciences, Pushchino, Moscow Region USSR	G	6000	Yes	distribution by exchange	VKM
Czechoslovakia National Collection of Type Cultures, Prague, Czechoslovakia	G	6000	Yes	free distribution to collections	IEM
Salmonella Genetic Stock Centre, Calgary, Alberta, Canada	G	6000	No	free distribution	LSCC
Canadian Collection of Fungus Cultures, Ste. Anne de Bellevue, Quebec Canada	G	5700	No	conditional distribution	CCFC
Deutsche Sammlung von Mikroorganismen, Goettingen, FDR	G	5000	Yes	fee for distribution	DSM
National Collection of Type Cultures, London, England UK	G	3800	Yes	fee for distribution	NCTC
National Collection of Industrial Bacteria, Aberdeen, Aberdeenshire, UK	G	3500	Yes	fee for distribution	NCIB
National Collection of Dairy Organisms, Reading, Berkshire, UK	G	3500	Yes	fee for distribution	NCDO
National Collection of Plant Pathogenic, Bacteria, Harpenden, Herts., UK	G	3000	Yes	conditional distribution	NCPPB

* Type of collection: I = Industrial corporation; G = Government; NP = Non-profit or not-for-profit.

Table 3. Microbial Culture Collections in the United States Identified by Type*

Reference & Archival	Education & Research	Industrial	Living Resource
NRRL (Peoria, IL)	DA (Ames, IA)	SCB (Bloomfield, NJ)	ATCC (Rockville, MD)
BRCC (Beltsville, MD)	FGSC (Arcata, CA)	LILLY (Indianapolis, IN)	
NBL (Berkeley, CA)	LMS (Burlington, NC)	UPJOHN (Kalamazoo, MI)	
NIAI (Bethesda, MD)	ACF (Columbia, SC)	SSI (Stamford, CT)	
VBDD (Fort Collins, CO)	TMC (Denver, CO)		
YARU (New Haven, CT)	ELSO (Dugway, UT)		
TAL (Paia, HI)	ADPH (Fairbanks, AK)		
PHLO (Portland, OR)	OIH (Waimanalo, HI)		
SFRE (San Antonio, TX)	AMC (Washington, DC)		
YARU (New Haven, CT)	WVDH (S. Charleston, WV)		
NRL (Seattle, WA)	MCT (Galveston, TX)		
PFRC (Stanford, CA)	SIO (La Jolla, CA)		
	IMRU (Piscataway, NJ)		

* acronyms from the World Directory of Collections of Microorganisms (McGowan and Skerman 1982)

APPENDIX I

Survey Instrument

Battelle
C.F. Kettering Research Laboratory
150 E. South College Street
P.O. Box 268
Yellow Springs, Ohio 45387
Telephone (513) 767-7271

Dear

Dwight Baker and I are doing a study on "Technologies to Maintain Microbial Diversity" as part of an overall project by the Office of Technology Assessment of the U.S. Congress on Technologies to Maintain Biological Diversity.

An assumption was made in the planning phase that microbial diversity is subject to genetic erosion and vulnerable to environmental dislocation in more or less the same way as appears to be the case with plants.

I questioned this assumption and as a result we got landed with the job of assembling evidence one way or the other!

We have found a few commentaries on the subject, but very few studies. In the absence of definitive studies, educated viewpoints of experienced professionals may be all that we have to weigh the case.

In view of the important role played by OTA reports in contributing to policy decisions impacting on U.S. Government legislation and appropriations, I thought you would welcome this opportunity to provide some input.

I attach the scope of our study so you can appreciate its purposes. Comment on any aspect is welcome.

My thinking at this time is that microbial genetic diversity, per se, is not substantially threatened. What seems to be in jeopardy is the investment to date in assembly of geographicallly diverse, characterized elite strains that may not be accessible to even current, let along future, users. So, much of our report deals with an institutional framework and management system that would allow future users of microbial diversity to capitalize on the investment of time and effort of their predecessors.

Our report will be fully referenced and any contributions you make and which we are able to use will be acknowledged.

Yours sincerely,

Jake Halliday
Director

JH:sgm

Attachment

Topics to be Addressed

1. Assessment of the state of knowledge of microorganism diversity and the extent to which diversity is increasing or decreasing.

2. Description of the different types of microbial collections that are used for reference, basic research, or industrial purposes with assessment of the vulnerability of the various collections.

3. Assessment of the existing and most promising future technologies to preserve microorganisms.

4. Assessment of the major technical constraints that limit the storage and use of microorganisms.

5. Discussion of institutional constraints or opportunities to improve coordination between repositories, or to improve use of the accessions.

6. Discussion of how the above constrains could be overcome with actions that institutions could take and their expected impacts.

7. Discussion of mechanisms through which Congress could encourage institutions to carry out the prescribed actions.

APPENDIX II

Survey Responses

Agracetus

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(608) 836-7300

July 22, 1985

Dr. Jake Halliday
Director
Battelle
C.F. Kettering Research Laboratory
P.O. Box 268
Yellow Springs, OH 45387

Dear Dr. Halliday:

Thank you for your letter of June 25 to Michael Goldberg describing your study on maintaining microbial diversity. He referred it to us for consideration.

We agree with your thinking as you expressed it in your letter, however, we don't have anything to add at this time.

I would look forward to seeing the results of your effort.

Sincerely,

AGRACETUS

Allen J. Dines
Director, Business Development

AJD:ch

IOWA STATE
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July 19, 1985

Dr. Jake Halliday, Director
C.F. Kettering Research Laboratory
150 E. South College Street
P.O. Box 268
Yellow Springs, Ohio 45387

Dear Dr. Halliday:

I apologize for the delay in replying to your letter of 3 June 1985 but I have been out of the office most of the time. Your project is a noble one and is of great importance to the field of microbiology.

I can comment especially on the matter of culture collections and feel strongly that the case for maintenance of strong collections needs to be made over and over to the government. To help you in your collection of data and ideas I would refer you to a slim book that was published in 1981 that succinctly answers many of the questions you have been asked to address. I am enclosing photocopies of the title pages of the book to help you obtain a copy if none is available in your library. If the book is out of print let me know and I will photocopy my copy and send it along. I think the report is valuable as a series of statements and recommendations for action on this issue. With it you would not have to "reinvent the wheel" and I won't have to compose a long polemic.

The conference in College Park was one of three held within a two year span that dealt with the topic. One of the others was sponsored by NIH on medically important collections and the other was sponsored by the USAID of the State Department. I do not know whether there were any formal reports that came out of either conference but you might check with the following people who might know: Dr. Micah Krichevsky, Building 31, Room 3B04, NIDR, NIH, Bethesda, MD, 20205, (Ph. 301/496-3652), for the NIH conference and Dr. Lloyd Frederick, Soil Microbiologist, USAID, ST/AGR, 420 SA-18, Washington, D.C., 20523, (Ph. 703/235-1275) for the USAID conference. These people can, at least, point you in the right direction.

Another source of ideas is the report of the 50th anniversary symposium of the ATCC (see enclosed). Obviously, much of what you need to convey to OTA has already been stated by others.

When one discusses the need to preserve microbial germ plasm one constantly runs into the argument that there is no need to preserve microbial cultures because one can go back to the original source and reisolate the organism. This is macrobiological thinking that has no basis in fact. Microorganisms are elusive; witness the unsuccessful attempts in the past to go back to the original source to reisolate type strains of important species of bacteria;

eg. *Flavobacterium aquatile*, *Achromobacter liquefaciens*, and *Bacterium triloculare*. The exact sources of the original strains were known but the organisms were never found in them.

I agree with you that microbial diversity is not threatened but collections of microorganisms are and that steps must be taken to maintain these collections.

I will be happy to help you in any way I can. Please let me know. Good luck.

Sincerely yours,

John G. Holt

John G. Holt, Professor

JGH:cdp

Enclosure

The Role of Culture Collections in the Era of Molecular Biology

Edited by RITA R. COLWELL

American Society for Microbiology
Washington, D.C.
1976

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National Work Conference on

Microbial Collections of Major Importance to Agriculture

Report of the Conference Convened at
the University of Maryland,
College Park, Maryland

March 12-14, 1980

Published by the American Phytopathological Society

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July 10, 1985

Dr. Jake Halliday
Battelle
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Ohio 45387

Dear Dr. Halliday:

Dr. Karuna-Karan asked me to respond to your letter of June 25 concerning your study on "Technologies to Maintain Microbial Diversity".

We greatly appreciate your invitation to participate in what I am sure is a major and time-consuming project. Unfortunately, we do not at this time have the spare manpower to do justice to the subject - our resources are highly limited and consequently we regretfully have to decline your invitation.

Sincerely,

Geoffrey Haigh, Ph.D.
Senior Scientist

THE PENNSYLVANIA STATE UNIVERSITY
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July 9, 1985

Dr. Jake Halliday
Battelle
C.J. Kettering Research Laboratory
150 E. South College Street
P.O. Box 268
Yellow Springs, Ohio 45387

Dear Dr. Halliday:

I have looked at the questions you have raised concerning microbial diversity. To some extent these questions cannot be answered, because there are more basic problems with which to contend. My reactions, somewhat rambling and not categorized by question, are as follows. I am considering mainly the organisms from the soil and aquatic habitats, and not necessarily those which are disease related.

There are many governmental, industrial and university culture collections. Bergey's Manual has a list of some of them. Most of these are checked for stability, with time, of the cultures. Many of these collections, however, continue to exist only because there is a current need for them, and because someone is paying for their labor-intensive and space-intensive requirements. By its very nature a culture collection should continue to increase in size. Often this is not the case, because organisms no longer of interest are deleted from the collection. Thus, many collections may continue to exist, but their contents may change to a small or large degree with time.

Many of the organisms in various culture collections have never been cross checked against other collections to determine if the organisms have been properly identified and named. In fact, I doubt that the holders of industrial collections would even allow this sort of thing.

There is a taxonomic problem concerning the question of maintenance of diversity. Are we talking about species diversity or strain diversity? Any one species may contain some to many diverse but genetically stable strains. Other strains of a species may not actually be different: only their plasmids are different.

Perhaps the best method to maintain microbial diversity would be to maintain a portion of the organisms' habitat so that the microbes could be retrieved at any future time: e.g., samples of freeze dried or frozen soil. Unfortunately, no one has evaluated the possible methods of doing this.

As regards habitat preservation, pure cultures of organisms that have not yet been isolated cannot be maintained in culture collections. I am referring to those organisms whose existence is not yet known. Most of the organisms (perhaps 99%) in soil and water have not yet been isolated or are ultra difficult to isolate. These fall into a group that has extreme difficulty in adapting to laboratory cultivation. *Agromyces ramosus* is a known example of this group, and its isolation is extremely difficult. We know that there are other organisms in this group. When we first isolated strains of *A. ramosus*, we simultaneously isolated representatives of several other new, numerically-dominant (in soil) types or organisms that fall into the "non-isolatable" group. However, we did not attempt to give them a taxonomic status.

The following are some observations:

1. Although we do not know the present extent of microbial diversity in nature, there has been no apparent major increase of decrease. Thus, we don't see any apparent ecological shifts that would be a result of this, nor any apparent loss of species.

2. Species added to nature usually die out and do not upset the ecology.

3. Upset ecologies seem to return to normal once the perturbing agent has been removed.

4. Some organisms in nature are present in only very small, virtually non-detectable numbers.

5. Microbes in nature are dormant much of the time.

6. The soils at the Rothemstead Experiment Station in England do not normally contain *Azotobacter*.

7. The pharmaceutical companies tell me that they isolate the same *Streptomyces* species from soil all over the world, and also from ocean muds.

I don't know if the foregoing is of much help. I am quite willing to answer more questions, or more specific questions, if you should so wish.

Sincerely yours,

L. E. Casida Jr. (Earl)

L. E. Casida, Jr.
Professor fo Microbiology

LEC/rab

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8 July 1985

Dear Jake,

Many thanks for your letter of 17 June. I am delighted to learn of your study on "Technologies to Maintain Microbial Diversity".

As concerns your second and fifth paragraphs of your letter, you are certainly bound to get the task mentioned in your third paragraphs.

Anyway, microbial diversity is subject to genetic erosion primarily for reasons of economy, overladen tasks, insufficient resources, etc. when dealing with endangered collections --much has been said and possibly very little done since none of the big collectio want to or can help.

I will write you later in detail, but for the moment am delighted to have made contact.

Yours sincerely,

Edgar J. DaSilva
Division of Scientific Research
and Higher Education

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In Reply Please Quote:

DEPARTMENT OF MICROBIOLOGY

HEAD: DR. J. G. ATHERTON
B.SC.APP., PH.D., M.A.S.M.

1st July, 1985.

Dr Jake Halliday,
Director,
Battelle,
C.F. Kettering Research Laboratory,
P.O. Box 268,
Yellow Springs, 45387
OHIO.

Dear Dr Jake Halliday,

I have been reflecting on your letter of May 24.

No doubt you've been in touch with Mort Starr of U.Cal . at Davis (now retired and I believe a Distinguished Professor at the University of Arizona). He seems to be the main advocate for diversity if he can get someone else to write it!

The only person that I have heard advance any thoughts on the erosion principle has been Dr Joan Knapp in relation to changes in the Neisseria group. Her address, (as I know it) is:

Dr Joan Knapp,
Neisseria Reference Laboratory,
Division of Infectious Diseases,
United States Public Health Hospital,
1131 14th Avenue S,
Seattle, Washington 98114,
U.S.A.

Then there is a liklihood that those dedicated to using antibiotics to suppress organisms in isolation procedures could contribute a little.

As for your "topics to be addressed". I have just one year and 4 days to retirement and more than enough to fill my time thank you!

I suggest you tax your American colleagues like Bob Stevenson at the ATCC and Rita Colwell at the University of Maryland. They are still young enough to find time enough to get round to an answer before they retire - and they would have more interest - in what the US Congress thinks or does. All it does for us is devalue our dollar to an all-time low.

Meanwhile I'll pass the letter onto the Chairman of the Australian Federation of Culture Collections who might be interested in helping.

Yours sincerely,

V.B.D. Skerman.

UNIVERSITY OF MARYLAND
COLLEGE PARK 20742

DEPARTMENT OF MICROBIOLOGY
AREA CODE (301) 454-5376

June 26, 1985

Dr. Jake Halliday, Director
Battelle
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150 E. South College Street
P. O. Box 268
Yellow Springs, Ohio 45387

Dear Dr. Halliday:

Thank you for your letter of May 24, 1985. I apologize for being a bit slow in replying. I was very interested in learning that you and Dwight Baker are doing a study on "Technologies to Maintain Microbial Diversity" as part of the overall project of the OTA.

Microbial diversity is not necessarily subject to genetic "erosion" but is vulnerable to environmental dislocation in much the same way as is the case with plants. Enclosed is a set of reprints describing research which we have done on microbial diversity in the Puerto Rico trench at the pharmaceutical dump site. I think you will find this study to be very helpful and definitive.

I hope that the information arrives soon enough to be included in your report.

Indeed, microbial genetic diversity is substantially threatened, especially when overwhelming changes in the environment are induced. As for example, in the case of the ocean dumping.

I will be happy to talk to you further.

Yours sincerely,

Rita R. Colwell
Professor of Microbiology

RRC:gw
Enclosure

American
Type Culture
Collection

12301 PARKLAWN DRIVE · ROCKVILLE, MARYLAND 20852-1776 USA · (301) 881-2600 · TELEX: ATCCROVE 908-768

June 20, 1985

Mr. Jake Halliday
Director
Battelle
C.F. Kettering Research Laboratory
150 E. South College Street
P.O. Box 268
Yellow Springs, Ohio 45387

Dear Mr. Halliday:

I have attached my comments on the topics you included in your letter.

There have been several conferences held in the Washington, D.C. area dealing with various aspects of the topics you listed. I have cited these reports in the event that you have not seen them. I have also enclosed a copy of a speech presented by Dr. Stevenson, Director of the ATCC, at the Third International Mycological Congress held in Tokyo, Japan.

One of the greatest problems facing the preservation of germplasm cultures is stabilized funding for these activities. In addition, there seems to be no national recognition that the preservation of microbial cultures represents a national resource.

I hope my comments prove helpful and if I can be of further assistance, please feel free to call me.

Sincerely yours,

Robert L. Gherna

Robert L. Gherna, Ph.D.
Head, Department of Bacteriology

RLG:[illegible]

Enclosures

AFFILIATED ORGANIZATIONS
AMERICAN ASSOCIATION OF IMMUNOLOGISTS · AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES · AMERICAN PHYTOPATHOLOGICAL SOCIETY · AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS
AMERICAN SOCIETY FOR CELL BIOLOGY · AMERICAN SOCIETY FOR MICROBIOLOGY · AMERICAN SOCIETY OF PARASITOLOGISTS · AMERICAN SOCIETY OF ZOOLOGISTS
AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE · CANADIAN FEDERATION OF BIOLOGICAL SOCIETIES · GENETICS SOCIETY OF AMERICA · INFECTIOUS DISEASES SOCIETY OF AMERICA
MYCOLOGICAL SOCIETY OF AMERICA · NATIONAL RESEARCH COUNCIL-NATIONAL ACADEMY OF SCIENCES · SOCIETY OF PROTOZOOLOGISTS · TISSUE CULTURE ASSOCIATION

The question of whether there is any genetic erosion due to environmental conditions is a complex one. There are very few studies, in my judgment, which deal with this subject in a comprehensive manner. There are undoubtedly "selections" of variants which are due to environmental shifts. An example of this situation is the appearance of organisms which are tolerant to or actually use pollutants such as mercury, cadmium, pesticides, etc. Whether environmental changes pose a threat to existing germplasm is hard to document. I would venture that there are some organisms that do exhibit genetic drift but whether the drift is a permanent one would be hard to determine in the absence of clear cut data. Studies have been conducted in the past which demonstrate population decreases or even changes due to various external (environmental) conditions. But these projects have dealt with whole populations and not with single species. A conference on the U.S. Strategy on Biological Diversity was held on November 16-18, 1981. The proceedings of the conference were published by the Department of State, Publication 9262 and were released April, 1982. The microbiological aspects are presented on page 86 of the proceedings.

Item 2. On March 12-14, 1980, a conference was held on Microbiological Collections of Major Importance to Agriculture. The report was published by the American Phytopathological Society in 1981 (Library of Congress Catalog # 81-66705). Many of the points brought up at the conference are applicable to collections in general which are valuable to industry, education, and health and human welfare. A description of various kinds of culture collection is presented on page 43 of the report. A majority of the problems brought up at the 1980 conference are still applicable today. Specifically the problems of funding collection activities as well as establishing a funded microbial data network are paramount concerns for culture collection specialists.

Culture Collections can be categorized by their functions and goals. The public repositories such as the American Type Culture Collection (ATCC) provide various services to a diversified assemblage of clientele worldwide. The ATCC, in essence, serves the scientific community as a "National Bureau of Microbiological Standards." I should emphasize, however, that although the name of our institution incorporates "American Type" there is no affiliation with any U.S. government agency. The ATCC is a nonprofit, private institution developed by scientists for scientists and as such it is constantly faced with monetary problems.

Other collections can be defined as specialist collections dealing with specific groups of microorganisms. For example these kinds of collections would consist of genetic organisms, certain taxonomic groups, clinical agricultural strains, etc. Many of these collections deal with a narrow range of clientele.

There are also research collections which maintain strains important to research projects or research institutes' programs. The specialist and research collections are often in danger of being lost when the interest of the curator/researcher shifts or upon their retirement.

Public collections abroad are mainly affiliated with government agencies or in the case of Japan with some fermentation industries. These governments support their collections' activities. In my judgment, there is no U.S. government recognition of microbiological germplasm as a national resource nor a central plan to finance it. In connection with the ATCC, it falls on each department to seek grant monies to finance various collection activities. The National Institutes of Health have provided generous support for the ATCC's activities but these are contract monies which must be negotiated at various time periods. There is no certainty that these funds will be forthcoming to finance culture collection activities.

From the scientific user's viewpoint, culture collections which house well authenticated cultures are invaluable. They serve as a reliable source of research material as well as standards for bioassays and other activities. It releases them from the need to maintain collections of their own to fulfill their needs. The preservation methods and quality control procedures of culture collections ensure the recipient that a culture is stable, free from genetic drift. Comparative studies can be made of earlier work with confidence.

The preservation techniques employed by culture collections are the state-of-the-art technology. I have not observed any culture which could not be preserved successfully using present day techniques and cryopreservatives. The preservation of microorganisms is an expensive proposition. We are discussing preservation in its most ideal form and not the continual transfer of cultures. Quality control procedures and redundant safeguards must be implemented and maintained. These include backup generators, liquid nitrogen tanks and freezers as well as a surveillance program for vital equipment. These safeguards ensure the long-term preservation of germplasm.

Item 5. The United States Federation for Culture Collections (USFCC) was estalished to enable people involved in culture collections as well as those interested in different aspects of culture collections to exchange information. The Federation publishes a newsletter and sponsors seminars germaine to culture collection work. The World Federation for Culture Collections (WFCC) functions in a similar manner but it is international in scope. Many federations such as the USFCC are members of the WFCC.

The participants of the two conferences cited above pointed out a need for a microbial strain data network. Ideally, a computer-strain data base would be most advantageous to microbiologists. There have been several international conferences sponsored by the United Nations to address the feasibility of establishing an international strain data network. A task force was established and exploratory work initiated.

It is important, in my opinion, for the U.S. government to realize the importance of national repositories as a resource and that the accompanying strain data generated by investigators be part of a data base. These data can serve many functions, aid biotechnology, located endangered collections and identify potential research strains or hazards such as the Salmonella outbreak in Chicago.

Robert E. Stevenson, American Type Culture Collection, Rockville, MD

THIRD INTERNATIONAL MYCOLOGICAL CONGRESS - AUGUST 28-SEPT. 3, 1983
TOKYO, JAPAN

FUNDING PROBLEMS OF CULTURE COLLECTIONS
NATIONAL & INTERNATIONAL

Reflexes and instinct are inherent properties of all life observed throughout the animal kingdom. One does not think about breathing or blinking, birds build highly species specific nests without plans or training. Likewise scientists collect things and usually don't think of this activity apart from their ordinary course of work.

Collecting is so taken for granted that usually no category of funds, levels of effort nor even significant attention is directed to collection activity when support is sought from granting agencies.

After years of work and accumulation of extensive reference collections, scientists are often dismayed to find that their university or their granting agency is surprised to be asked to provide a home for their material and reluctant to agree.

Administrators should know of this collecting propensity of scientists only if they are scientists themselves - most are not and therein lies the first level of funding problems.

The second level funding problem has to do with the exchange of "courtesy" or gratis cultures between scientists.

This form of professional courtesy, a benevolent generosity, can also be defined as charity - the rich giving to the poor. Although this practice is considered a virtue in most cultures - establishing a type of moral savings account - it can have side effects that are pernicious.

We have a saying - "Don't look a gift horse in the mouth"-. In this case of gift cultures you are foolish not to do so because what you get may not be a horse but a dragon. Unless you independently verify it or have it done for you by competent authority, years of work and lots of money can be wasted studying the wrong thing.

THIS TRAFFIC IS MOST ACTIVE IN NEWLY DESCRIBED MATERIAL. THE INVESTIGATOR ORIGINATING THE MATERIAL IS FLATTERED BY THE ATTENTION AND OFTEN SPENDS CONSIDERABLE RESOURCES TO COMPLY WITH THE REQUESTS. THE RECIPIENT, FEELING HE HAS THE MEANS TO BE *AU COURANT* AND AHEAD OF THE PACK [redacted] IS APT TO OVERLOOK TESTS FOR IDENTITY AND PURITY THAT WOULD REALLY DEFINE WHAT HE HAS.

THIS SERVICE CAN ALSO BE A FINANCIAL BURDEN BOOTLEGGED OUT OF THE DONOR'S GRANT FUNDS AND CERTAINLY IS A DISTRACTION FROM HIS ORDINARY RESEARCH.

CUSTOMARILY NO CHARGE IS LEVIED FOR THIS COURTESY AND THERE SEEMS TO BE A CONSPIRACY OF SILENCE TO SHIELD THIS FACT FROM ADMINISTRATIVE PEOPLE WHO PAY THE BILLS. SOMEHOW THIS MUST BE CHANGED AND THE PRACTICE LEGITIMIZED.

THIS IS A NORMAL COST OF DOING RESEARCH AND SHOULD BE RECOGNIZED AS SUCH.

THE THIRD LEVEL OF PROBLEM IS THE FINANCIAL SUPPORT OF REFERENCE COLLECTIONS AND NATIONAL REPOSITORIES. MICROBIAL COLLECTIONS ARE NOT UNIQUE IN HAVING THESE PROBLEMS BUT ZOOLOGICAL AND BOTANICAL COLLECTIONS CAN PANDER TO THE PUBLIC'S CURIOSITY AND FINANCIAL SUPPORT BY EXHIBITING THEIR SPECIMENS AND PROVIDE A FORM OF CULTURAL ENTERTAINMENT. MONKEYS ARE CUTE AND WE SEE OUR FOIBLES REFLECTED IN THEM, BUT, TRY AS WE MAY, IT IS HARD FOR HUMANS TO IDENTIFY WITH A *MUCOR* OR *PENICILLIUM.*

WE HAVE NOT DONE A GOOD JOB OF INFORMING THE PUBLIC AND ITS POLITICAL REPRESENTATIVES OF THE IMPORTANCE AND NEED FOR MICROBIAL COLLECTIONS AND UNTIL WE DO WHATEVER PUBLIC FUNDS WE OBTAIN WILL BE SMALL IN AMOUNT AND CAPRICIOUS IN COMING. KEEP IN MIND THAT SUCH REQUESTS ALSO CARRY THE OBLIGATION TO CIRCUMSCRIBE THE LIMITS OF COLLECTIONS.

BIGGER IS NOT NECESSARILY BETTER AND A WELL CULLED SELECTION OF REPRESENTATIVE STRAINS IS BETTER THAN MANY DUPLICATES VARYING ONLY IN PLACE AND TIME OF ISOLATION.

A COLLECTION REQUIRES A CONSTITUENCY OF USERS TO DEVELOP SUPPORT AND IT IS INCUMBENT UPON CURATORS TO PROVIDE USEFUL SCREENED MATERIAL NOT A GARBAGE DUMP.

THE FOURTH PROBLEM LEVEL IS THE LACK OF INTERNATIONAL ATTENTION TO THESE COLLECTIONS AND THE DEVISING OF MEANS TO ASSURE UNIFORM QUALITY AND READY WORLD WIDE ACCESS TO THE MATERIALS. THERE IS A TENDENCY FOR NATIONAL COLLECTIONS TO BE ISOLATED BECAUSE OF LOW FUNDING LEVELS. THIS INHIBITS CONFERENCES AND WORKING COMMITTEES FROM MEETING AND WORKING OUT PROCEDURAL SPECIFICS, DEVELOPING STRAIN DATA BANK NETWORKS, AND ESTABLISHING PROGRAMS TO DEVELOP AND EXCHANGE COLLECTIONS OF SCOPE AND DEPTH.

WHAT PALTRY RESOURCES ARE AVAILABLE ARE NOW BEING SPREAD VERY THIN AND IN MY OPINION A LOT OF DUPLICATION OF EFFORT IS SQUANDERING MONEY BETTER SPENT ON ORGANIZED COLLABORATION.

IT IS OFTEN SAID THAT DEFINING THE PROBLEM IS NONE THAN HALF OF THE SOLUTION TO IT.

THERE IS ALSO AN OBLIGATION FOR THE CRITIC TO SUGGEST WAYS TO IMPROVE WHAT IS FOUND DEFICIENT.

THE FIRST LEVEL PROBLEM - RECOGNIZING THE COLLECTING PROCESS AS INTEGRAL TO THE PURSUIT OF SCIENTIFIC KNOWLEDGE IS OBVIOUS BUT NOT TRIVIAL.

EDUCATIONAL PROCESSES MUST BE INVOKED AND INCLUDE CHAPTERS IN TEXTBOOKS, INFORMATIVE ARTICLES IN SCIENTIFIC AND LAY MEDIA AND EXPOSURE OF SCIENTIFIC ADMINISTRATORS TO THE CONCEPT THAT COLLECTIONS ARE LIBRARIES AND DESERVE SIMILAR SUPPORT.

THE SECOND LEVEL PROBLEM - EXCHANGE OF "FREE" CULTURES WITH THEIR ATTENDANT RISKS COULD BE MINIMIZED BY MORE TIMELY AND AGRESSIVE ACCESSIONING OF NEW MATERIAL BY CURATORS. PROMPT AND THOROUGH CHARACTERIZATION BY EXPERTS BEFORE WIDE DISTRIBUTION WOULD INSURE THE SUBSEQUENT INVESTMENTS AND WOULD RELIEVE INVESTIGATORS OF NUISANCE AND FINANCIAL BURDENS.

THE THIRD LEVEL PROBLEM - INSTITUTIONAL SUPPORT FOR COLLECTION ACTIVITIES IS PRIMARILY A POLITICAL ONE. PUBLIC FUNDS ARE USUALLY MADE AVAILABLE IF WIDESPREAD SUPPORT FOR AN INSTITUTION IS PERCEIVED BY GOVERNMENT. TO THIS END SCIENTISTS THROUGH THEIR SCIENTIFIC SOCIETIES NEED TO DEFINE AND PROMOTE THE USEFULNESS OF THESE LIBRARIES AND THE ECONOMIC SENSE OF SHARING THESE RESOURCES RATHER THAN HAVING TO MAINTAIN MULTIPLE SMALL EFFORTS OR TO DEPEND UPON CONTINUAL RE-ISOLATION OF MATERIAL. THE VALUE OF NATIONAL OR INTERNATIONAL COLLECTIONS DEPENDS UPON REFERENCE COLLECTIONS DEVELOPED BY PEER SPECIALISTS AND THOSE IN TURN DEPEND UPON THE INDIVIDUAL SCIENTIST'S CREATIVITY AND INITIATIVE. THIS PROCESS CONTINUENCE NEEDS TO BE DEFINED, EXPLAINED AND RATIONALIZED TO A MUCH LARGER AUDIENCE THAN HERETOFORE DONE.

THE FOURTH LEVEL PROBLEM - BETTER INTERNATIONAL COOPERATION AND USE OF RESOURCES IS APPROPRIATELY ADDRESSED IN A FORUM SUCH AS TODAY'S MEETING. REPRESENTATIVES OF MAJOR COLLECTIONS AND INDIVIDUAL SCIENTISTS CAPABLE OF CONTRIBUTING TO THESE EFFORTS ARE HERE. FIRST, A CULTURE COLLECTION COMMITTEE AS A STANDING COMMITTEE NEEDS TO BE APPOINTED AND CHARGED WITH RESPONSIBILITY TO INVENTORY AND EVALUATE COLLECTION ACTIVITIES. NATIONAL AND REGIONAL COLLECTIONS AND FEDERATIONS OF COLLECTIONS NEED TO BE INVOLVED IN THIS PROCESS SUMMARIES AND RECOMMENDATIONS FOR IMPROVEMENT OF INTERNATIONAL EXCHANGES AND STANDARDS NEED TO BE DEVELOPED AND FORWARDED TO APPROPRIATE INDIVIDUALS AND INSTITUTIONS FOR POLICY AND FNIANCIAL SUPPORT.

COMING FROM AN INTERNATIONALLY RECOGNIZED ORGANIZATION OF SCIENTIFIC PEERS AND SOCIETIES SUCH A STATEMENT WOULD HAVE AN EFFECT ON POLICY DECISIONS.

THE PRECEDING RECOMMENDATIONS DISTILL THE THOUGHTS AND OPINIONS OF A NUMBER OF CONFERENCES, ADVISORY PANELS AND INDIVIDUALS. I CLAIM NO CREDIT FOR UNIQUE INSIGHTS BUT FEEL VERY PASSIONATELY THAT NO PROGRESS WILL BE MADE UNTIL WE COLLECTIVELY ACT IN A POSITIVE WAY TO IMPROVE MATTERS.

COLLECTIONS OF THE WORLD - UNITE.

THE STATE UNIVERSITY OF NEW JERSEY
RUTGERS

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June 10, 1985

Dr. Jake Halliday, Director
Battelle
C. F. Kettering Research Laboratory
P. O. Boxx 268
Yellow Springs, OH 45387

Dear Dr. Halliday:

Your letter of June 3, 1985, to Dr. David Pramer was turned over to me.

Your list of topics to be addressed concerning microbial diversity is interesting. We should remember that microbes include bacteria, fungi, algae, protozoa and viruses. In each group there is much diversity and, with the passage of time, there is also an apparent increase in diversity which is probably due more to our increase in our knowledge of these organisms than to an actual increase in diversity in nature.

Dr. V. Skerman, c/o The Librarian, University of Queensland, St. Lucia, Brisbane, Australia, would be the best person to discuss microbial culture collections.

Cold storage, including liquid nitrogen, freeze drying and cultures in sterile soil, seem to be the best methods of preservation for microbial cultures. Future methods might aim at preserving only the genetic material.

Space limitation and lack of financial support are probably the major technical constraints limiting storage of microorganisms. As far as the use of microorganisms is concerned, we are often limited by our lack of knowledge on how to grow microorganisms.

Congress could help in preserving microbial diversity by increasing support to the American Type Culture Collection and the culture collection of the Northern Regional Research Laboratories of the Department of Agriculture. In these and other institutions, increased funding should be earmarked to support the research of those who try to learn how to grow microorganisms. Why are there organisms that we cannot grow? Why are there slow-growing and fast-growing organisms? What could we do to change metabolic programming from slow to fast?

Please do not hesitate to contact me if you feel that I can be of further assistance.

Sincerely yours,

H. Lechevalier

Hubert A. Lechevalier, Ph.D.
Professor of Microbiology

HAL/cw

7

New York State College of Agriculture and Life Sciences
a Statutory College of the State University
Cornell University

Department of Agronomy
Bradfield and Emerson Halls, Ithaca, N.Y. 14853

June 5, 1985

Dr. Jake Halliday, Director
Battelle
C. F. Kettering Research Laboratory
150 E. South College Street
P. O. Box 268
Yellow Springs, OH 45387

Dear Jake:

I really have given little thought to the question of maintaining microbial diversity. However, the few studies that have been done are worth citing. For example, Ruth Patrick, who is still an active scientist in Philadelphia, carried out a number of studies on stresses affecting microbial diversity (for example, Annals of New York Academy of Sciences, 108:359-365, 1963). It might be worth writing to her to get an update of her studies on diatoms.

I agree that it is unlikely that microbial genetic diversity is under threat. However, what is not being adequately supported is the maintainance of culture collections of microorganisms, not only the elite strains such as those mentioned in your letter, but also a wide variety of microorganisms of theoretical and practical interest.

Sincerely yours,

Martin

M. Alexander, Professor
Soil Microbiology

dm